MR Tracking of I.V. Injected Magnetically Labeled Mesenchymal Stem Cells (MSCs) in a Rat Model of Nephropathy.

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Introduction

Renal insufficiency is a major problem in public health. Many renal diseases involving tubular and glomerular cells are irreversible and progressive, leading to acute or chronic renal failure. Specific therapies are missing in most of these nephropathies and very little is known about the prospect of using stem cells in their treatment. A few studies showed the ability for bone marrow-derived cells to differenciate into glomerular mesangial cells (1,2) and to deliver a modified phenotype to normal glomeruli (3). Using superparamagnetic iron oxide (SPIO) to magnetically label cells, MR imaging has shown great promise as a noninvasive method to monitor experimental cell-based therapies (4). The objective of the present study was assess in-vivo and ex-vivo imaging of i.v. injected magnetically labeled mesenchymal stem cell for repair of mesangial glomerular cells in an experimental model of mesangiolysis.

Methods

Animal model: Persistent glomerular perturbation with prolonged phenotypic change of mesangial cells was induced by concomitant injection 1mg/kg of OX7 antibody and 60mg/kg of aminonucleoside of puromycin. Cell labeling: MSCs from Lewis rats (kindly provided by Dr. D. Prockop, Tulane University) were grown for 48 hours in modified medium containing 2.2µl/ml of dextran-coated SPIO MR contrast ferumoxide (Feridex; Berlex) and 0.25 µl/ml of Superfect (Qiagen) (5). One hour prior to injection, cells were labeled with a fluorescent membrane solution (DiI,Vybrant, Molecular Probes).

Animal experiments: This study involved 14 Lewis male rats (150-250g) divided into four groups: two pathologic groups (n = 5 each) in wich MSCs were injected respectively 4 and 8 days after the induction of the nephropathy; and two control groups (n = 2 each). In the first control group, the nephropathy was induced but no cells were injected and in the second control group cells were injected in normal animals. The number of MSCs injected in the tail vein of the rat varied from 8 to 10 millions. Animals were sacrificed 6 days after the MSC injection. For all experiments, animals were anesthetized with isoflurane. At time of sacrifice, animals were thoroughly perfused and both kidneys and liver removed. One kidney was fixed in 4% PFA for ex-vivo imaging and the other was embedded in OCT compound for cryosectioning.

In-vivo MR imaging eas performed before and 6 days after MSC injection on a 4.7T spectrometer (Bruker). A FLASH gradient-echo sequence (repetition time msec/echo time msec/ flip angle = 300/12/30) was used to enhance the T2* effects of the intracellular iron oxide particles. Six signals were acquired per image, matrix size of 256 x 256, field of view of 55mm, section thickness of 1mm, and imaging time of 7.40 minutes. Signal intensity was measured before and 6 days a after MSCs injection by placing ROI in each kidney compartment and on the liver.

Ex-vivo MR imaging: MR images were obtained using a 400 MHz (9.4T) GE Omega NMR spectrometer. A home made solenoid RF coil was used as both receiver and transmitter. A T2*-weighted gradient echo sequence was used with TR = 110 msec, and 4 echoes were obtained with a TE of 7.5, 15.7, 23.9 and 32.1 msec. The reconstructed matrix was 512 x 240 x 192 with the obtained resolution of 43 x 63 x 68µm.

Histology: OCT embedded cryosections were used for alphaactinand Perls staining and DiI labeling.

Results

In-vivo imaging: Qualitative visual analysis and signal intensity measurements of pathologic kidneys did not show any signal drop, particularily in the cortex ($p = 0.7$). On the contrary, a significant signal decrease was noted in the liver ($p<.01$) indicating the trapping of a significant number of labeled cells by the liver, acting as a filter.

Ex vivo imaging: All pathologic kidneys showed focal cortical (where glomeruli are located) areas of signal loss (Fig 1). Control kidneys did not show any signal change indicating the absence of impact of the nephropathy alone or the labeled MSCs alone on the signal of the kidney.

Histology : Anti-alpha-actin immunostaining showed a focal nephropathy with areas of glomerular damage while others were subnormal. Fluorescent analysis evidenced DiI positive MSCs cells in the focal areas of glomerular damage (Fig.2). The presence in the glomeruli of labeled MSCs was also confirmed by Perls staining (Fig.3). No MSCs were found in the control kidneys.

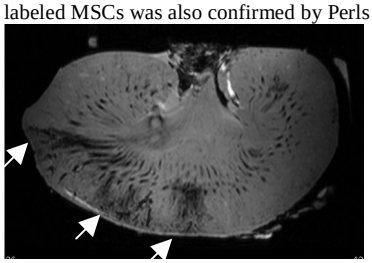

Figure 1: Sagittal T2*-weighted ex-vivo MR-image of a rat kidney 6 days after labeled MCS injection. Focal areas (arrows) of signal drop.

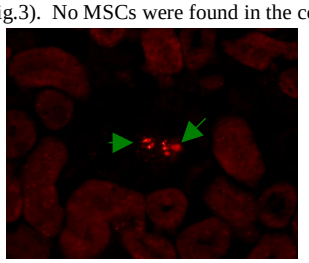

Figure 2: Mesenchymal stem DiI+ cells (arrows) homing into the glomeruli of the pathologic kidney.

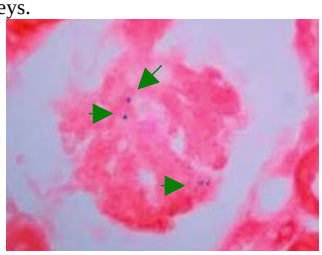

Figure 3: Renal glomeruli containing Perls positive labeled cells (arrows).

Discussion

Our initial results show for the first time, in this model of glomerular damage, that labeled MSCs injected intravenously can specifically localize in damaged components (glomeruli) of pathological kidneys and that these cells can be detected *ex-vivo*. This could be of great interest for cell-based therapies. However, the intravenous way seems limited by a major cell trapping in the liver and the impossibility so far to detect the cells *in- vivo*. The fate of the MSCs in the glomeruli has yet to be studied.

References

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