In-vivo MR monitoring of magnetically labeled mesenchymal stem cells in kidneys after selective intrarenal injection in a glomerulonephritis model in rats at 3T

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Introduction

Early chronic kidney disease and end stage renal failure is a major clinical and socio-economic problem. The current therapeutic opportunities to slow the progression of the disease are limited. Pluripotent stem cells, and especially mesenchymal stem cells (MSC), seem to have therapeutic potential in repair, revascularisation and regeneration of tissues in numerous genetic, degenerative or malignant diseases [1]. In renal diseases, MSC may have therapeutic potential to repair damaged renal structures or to improve kidney function [2,3]. In-vivo monitoring of cells with MRI after magnetically cellular labeling procedures was shown by several groups [4-6].

The purpose of this study was to detect Superparamagnetic Particles of Iron Oxide (SPIO)-labeled mesenchymal stem cells of the rat after selective injection in the renal artery and to monitor their in-vivo distribution with MRI at 3T using an experimental rat model of glomerulonephritis.

Material and Methods

For in-vitro cell labeling rMSC of Sprague Dawley (SD) rats were incubated with Resovist® (Schering AG, Berlin, Germany) for 24 h and cellular uptake was proven cytologically by Prussian blue staining. In 5 male SD rats (250.8 ± 5.5g body weight) a Thy-1 glomerulonephritis was induced by intravenous (i.v.) injection of anti-Thy 1 monoclonal antibodies. 4 days after induction of glomerulonephritis two groups with catheter-guided, selective intraarterial injection of 1×10^6 rMSCs (MSC group, n=3) and pure saline injection (control group, n=2) were built. MRI scans were performed 6 days before (baseline), 1-2 hours, 4, 7, 11 and 22 days after stem cell or saline injection. MR Imaging and Relaxometry was performed on a clinical 3T MR Scanner (Philips Intera) using a custom-made small animal solenoid coil. MRI data were acquired using a T2*weighted 2D gradient echo sequence (TR/TE 224/4.6ms, flip angle 80°, Field of View (FoV) 100 × 75 mm, Matrix 512×228, NSA 6, slices 12, slice thickness 1.5mm, effective voxel volume 20×33×1500µm). Relaxometry data were obtained by using a fat-saturated 3D multi gradient-echo sequence (14 odd echoes, inter echo time 4.2 ms, TR 100ms, flip angle 30°; FoV 85×76.5 mm, Matrix 128×128, NSA 1, slices 10, slice thickness 2mm, effective voxel volume 66×71×2000µm). T2* was calculated voxel-based using a software tool (RelaxFit, Philips Research Laboratories Hamburg, Germany). SNR were measured in kidneys, liver and spleen at each time point. Relative signal intensity (RSI) was calculated in relation to baseline as follows:

$RSI_{follow\ up}(\%)=SNR_{follow\ up}/SNR_{baseline}\times100$. Significant differences in SNR between MSC and control group were tested by unpaired student's t-test with Bonferoni-Holm corrected p-values (adjusted $p<0.05$). After euthanasia MR data were correlated with histology (Prussian blue).

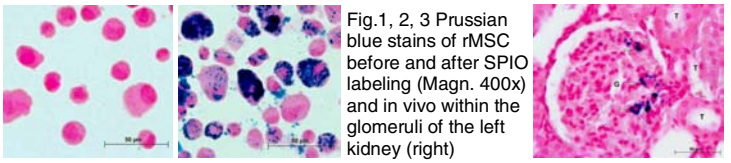

Fig.1, 2, 3 Prussian blue stains of rMSC before and after SPIO labeling (Magn. 400x) and in vivo within the glomeruli of the left kidney (right)

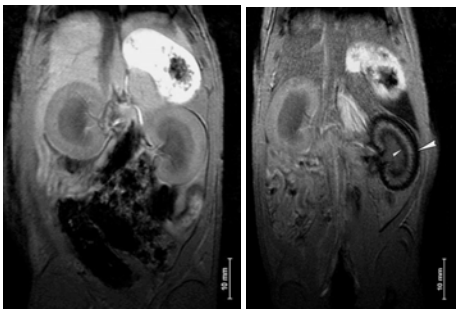

Fig.4 T2*w MR Images of rat kidneys before and after selective injection of SPIO-labeled rMSC in left kidney with rMSC in the glomeruli of the renal cortex and the outer medulla (white arrows)

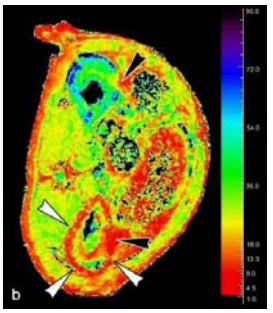

Fig.5 T2* map with significant T2* reduction in left kidney (white arrows) and artificial T2* shortening by bowel gas (black arrows)

Results

In-vitro incubation of rMSC with Resovist showed effective cellular uptake (Fig.1 and 2). Selective injection of labeled rMSC resulted in a significant signal loss in kidney of injection side (left) in T2*w MR images up to 3 weeks after application (Fig.4) in comparison to saline control group (adj. $p<0.05$). No significant changes in SNR in kidneys of saline control group could be detected ($p>0.05$), indicating, that nephropathy alone had an influence on kidney SNR (Fig.6). Liver and spleen showed a delayed maximum of SNR reduction (day 3-7), indication a prolonged accumulation of rMSC (Fig.7). Corresponding to MR Imaging T2* relaxometry data showed a significant decrease in left kidney (Fig.5) caused by presence of SPIO-labeled rMSC. According to MRI histology (Prussian blue) showed a predominance of iron oxide labeled rMSC inside the glomeruli of perfused kidney after cell administration (Fig.3).

Discussion and Conclusions

In this study we demonstrate the ability of serial in-vivo imaging of magnetically labeled stem cells (rMSC) in an animal model of glomerular disease in a clinical whole body 3T MRI scanner. We have shown the persistence of rMSC in glomeruli after intrarenal perfusion in MRI for at least 3 weeks after cell administration in correlation to histology and have indicators for a delayed migration to liver and spleen. Using MR Relaxometry, an initial approach for the quantification of labeled cells in tissue was given. Our preliminary study demonstrates the potential of MRI for a non-invasive in-vivo monitoring of magnetically labeled cells and offers the possibility of monitoring cell-based therapies in therapeutic treatment models in the future.

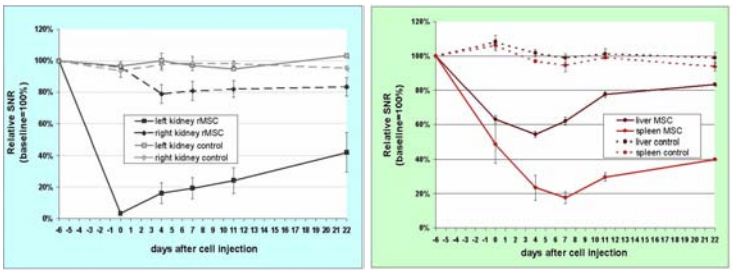

Fig 6, 7 Relative Signal intensities of kidneys (left), liver and spleen (right) in comparison to baseline (100%)

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