Intravascular Injection of Magnetically Labeled Mesenchymal Stem Cells in Kidney and Liver: *In vivo* MR Imaging Results

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Introduction

Bone marrow derived Mesenchymal Stem Cells (MSC) are presently being studied in many experimental cell therapy protocols, aimed at e.g. regeneration of the infarcted myocardium. MSC have been demonstrated to also colonize other organs including liver and kidney after grafting (1). There is a high likelihood that MSC could differentiate into hepatic stellate and renal mesangial cells (2,3) that are cells of mesenchymal origin. Hence, MSC are potentially important vectors for cell and gene therapy purposes in the liver or the kidneys.

Local intravascular administration of stem cells would be attractive to achieve distribution in a whole organ, which would be important for cell therapy applications in diffuse diseases, especially for organs like the kidney or the liver which are clinically accessible via the intra-vascular route. The purpose of this study was to demonstrate the ability of *in vivo* MRI on a conventional 1.5-T scanner to detect magnetically labeled MSC after local intravascular injection.

Methods

Cells: MSC were obtained from the bone marrow of Lewis 1A rats. Cells were labeled by incubation for 48 hours with an SPIO preparation (Endorem, Guerbet Group) at 50 µg Fe/mL and 0.01 % Superfect (Qiagen) (4).

Animal experiments: Healthy 300-gram Lewis 1A rats were injected in the left renal artery with $5\text{-}6\cdot10^5$ labeled (n = 4) or unlabeled (n = 2) cells using a 30-G needle. Rats were imaged before MSC-injection and directly after MSC-injection and after 2,4 and 7 days. Rats were sacrificed ultimately after 7 days or sooner when the signal drop on T_2^*-weighted images had nearly completely faded. A second group of 180-gram Lewis 1A rats with a CCl_4 hepatocyte necrosis model were injected $5\cdot10^6$ labeled (n = 2) or unlabeled (n = 2) MSC in the portal vein, two days after CCl_4-administration. These rats were imaged before and after MSC-injection, and after at 4, 8 and 12 days after MSC-injection. At day 12 rats were sacrificed. Kidneys or liver were removed and subjected to *ex vivo* MRI. Then, samples were fixed in 4% PFA and embedded in paraffin for histology. For all manipulations, rats were anesthetized with 0.5 mL 8% w/w per 100 g bodyweight chloral hydrate intra-peritoneally.

MR-imaging and image analysis: All imaging was performed on a 1.5-T MR-system (Gyroscan Intera, Philips, NL) The *in vivo* MR-imaging protocol consisted of fat-suppressed T_2^*-weighted multislice and 3D gradient echo sequences and a quantitative multislice multi-gradient echo technique. Liver or kidney were imaged using a 47-mm surface coil. The *ex-vivo* imaging protocol consisted of a T_2^*-weighted 3D scan. R_2^* was determined from multi-echo gradient echo sequence data by an exponential fit to the signal magnitude. In the kidney, R_2^*-values were determined in the renal cortex. In the liver, R_2^*-values were determined in the median and right lobe of the liver.

Histology: paraffin embedded serial microsections were used for Perls' staining and immunostainings for smooth muscle-α-actin in the kidney and CD90 in the liver.

Results

After injection of labeled MSC in the renal artery, a distinct signal loss on T_2^*-weighted imaging was observed in the renal cortex (Fig 1), that faded over time. No signal loss was observed upon injection of unlabeled cells. After injection of labeled MSC, R_2^* in the cortex changed from 17.7±1.8 to 35.3±8.3 s^{-1} on average (± SD). The evolution of R_2^* in the renal cortex on serial MR examinations (Fig 2), is in accordance with a fading of the signal loss on serial T_2^*-weighted images. The first rat that was injected in the renal artery was to be imaged at day 4,8 and 12, however, signal in the kidney went back to normal at day 4. With the tighter follow-up schedule, two rats showed an important fading of the signal loss at day 2, and were sacrificed. In one rat, signal loss persisted for 7 days.

Histological analysis showed Perls positive cells primarily in glomeruli in the area where a signal drop was observed (Fig 3). Immunostaining identified the Perls positive cells as MSC. Some MSC were localized in the interstitial tissue and under the renal capsule in the injected territory, no MSC were found in the non-injected vascular territory and contralateral kidney.

After intra-portal injection (no data shown), labeled MSC induced a diffuse signal loss in the entire liver on T_2^*-weighted imaging. The signal loss diminished but was still present at day 12. The liver signal had a granular appearance, indicating an inhomogeneous distribution of cells related to the lobular structure of the liver. After injection R_2^* increased from 32.5±0.4 to 56.0±4.6 s^{-1} (± SD). In rats injected with labeled MSC, histology showed Perls positive cells throughout the liver parenchyma. MSC tended to be localized along sinusoids and, to a lesser extent, near centrolobular veins and portal tracts.

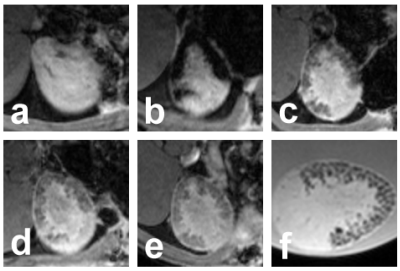

Figure 1: Transverse T_2^*-weighted MR-images of the rat kidney. (a) Before injection. (b) Within one hour after injection. (c,d,e) Serial images obtained after 2,4 and 7 days. (f) *Ex vivo* MR-image at 7 days.

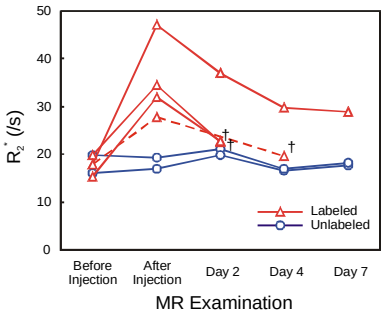

Figure 2: Serial R_2^*-measurements of the renal cortex after MSC injection. (†) Indicates sacrifice before day 7.

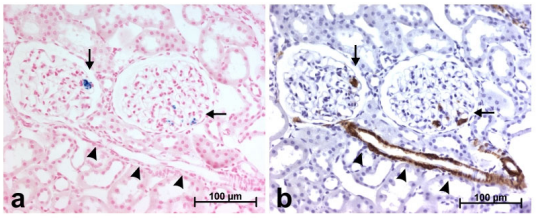

Figure 3: Kidney tissue sections at 7 days post MSC injection. Renal glomeruli containing (a) Perls positive cells (arrows), corresponding to (b) SMA positive cells. Glomerular arterioles (arrowheads), which contain vascular smooth muscle cells, are constitutively positive for SMA.

Discussion

Magnetically labeled MSC can be detected in rat kidney and liver by *in vivo* MR-imaging, using a 1.5-T MR-system within clinically acceptable imaging times. For the first time, it was shown that cells can be visualized after local intravascular administration, and that this route can be used to seed cells throughout the target organ. The technique has utility for developing and evaluating transplantation procedures used in future cell therapy protocols. It provides a means to immediately verify if the cells have indeed grafted within the target organ. Possibly, it may allow an estimation of the number of cells that were seeded. Finally, sequential imaging studies will allow to assess the permanence of the grafted cells over time.

References

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