Gadofluorine M uptake in stem cells as a new tracking method at 1.5T MRI

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Introduction

Cell tracking using with ultra-small iron particles is well established in MRI. However, in experimental models intrinsic iron signals derived from erythrocytes mask the labeled cells. Therefore, we evaluated Gadofluorine M versus other gadolinium chelates for a T1-weighted positive enhancement for cell tracking *in-vitro* and *in-vivo*.

Methods

Gadofluorine M and other gadolinium chelates were used to label stem cells with and without uptake-mediating agents using 1.5T-MRI scanner *in-vitro* and *in-vivo*. Additionally, histology and molecular modeling was investigated.

For all experiments mesenchymal stem cells (MSC) were derived from adipose tissue and the uptake procedure with Lipofectin was performed as followed: 100 µl Lipofectin was dissolved in 100 µl Dulbecco´s Modified Eagles Medium (DMEM, Cambrex BioScience, Verviers, Belgium). In separated tubes, Gd-DTPA, Gadomer or Gadofluorine M (Schering AG, Berlin, Germany) were dissolved in 100 µl DMEM. Both tubes were mixed and filled with DMEM to a final volume of 1 ml and incubated at 37°C / 5% CO_2 for 45 minutes. The cells were washed twice with phosphate buffered saline (PBS) before these contrast agent solutions were added. Regular MSC growth medium (MSCGM, Cambrex BioScience, Verviers, Belgium) without antibiotics was added to the cells, according to the recommendations of the manufacturer of Lipofectin. Preparation of the contrast agent solutions for cell labeling without Lipofectin was achieved as follows: Gd-DTPA, Gadomer or Gadofluorine M were dissolved in 1ml DMEM and incubated at 37°C / 5% CO_2 for 45 minutes. Afterwards, cells were further processed and incubated as described above. After adding contrast agent solutions to the cells a constant Gadolinium concentration of 0.25 nmol/L was used in all experiments.

In-vitro experiment:

MRI was performed on a 1.5T clinical scanner (Symphony, Siemens AG, Erlangen, Germany) using a dedicated head coil with a T1-w sequence (Spin-Echo, FA=90°, TR=540 ms, TE=12 ms, SD=1.9 mm, SL=3 mm). For the MRI examinations the probes were placed in a waterbath with a constant temperature of 37°C. Measurement of signal intensity was performed using the scanner software workstation with a standard region-of-interest of 2.5 mm^2 placed in the center of the cell pellet.

In-vitvo experiment:

Two adult male Wistar rats (Charles River, Sulzfeld, Germany) were anesthetized with isoflurane and placed in a stereotactic frame (David Kopf Instruments, Tujunga USA). Implantation of $1x10^6$ cells triple labeled with Gadofluorine M-Cy3.5 and BrdU (Sigma-Aldrich, Steinheim, Germany) for combined MRI and histological tracking was performed as followed: through a small hole drilled into the skull, a 26-gauge needle was slowly introduced into the right caudate nucleus. The cells dissolved in 30µl PBS were infused over 30 minutes using a CMA/100 microdialysis pump (CMA Microdialysis, Solona, Sweden). The same amount of cells without Gadofluorine M but labeled with BrdU were implanted on the contralateral side using the same implantation protocol. BrdU pre-labeling of the cells was performed in advance with an incubation of 10µM BrdU for 72h. After suture, animals were placed in a cage with free access to food and water.After 24h the animals were anesthetized with ketamin/xylazin and placed in a 1.5T MR clinical scanner (Siemens Avanto, Erlangen, Germany) and the neurocranium of the animals was imaged using a T1-Vibe (TE=7.2ms; TR=14.5ms) and T2 –multi-slab3D-TSE (TEeff=84ms; TR 3500ms; TurboFaktor 9) in conjunction with a 4 Channel High Resolution Wrist Array – coil (Invivo, Orlando, USA).

Results

Gadofluorine M revealed comparable properties to an uptake mediating agent in molecular modeling. Without an uptake-mediating agent Gadofluorine M labeled cells were detected as a T1-weighted positive contrast in-vitro and in-vivo. Histology confirmed a 100% success rate for intracellular labeling.

Cell labeling with various contrast agents

However, using the same gadolinium concentration for all contrast agents with and without uptake mediating agent (Lipofectin) there is a superior signal increase of the Gadofluorine M labeled cells. Comparison of signal intensities reveals only minor differences between cells treated with the combined mixture of gadolinium chelate and Lipofectin and the cells treated with the gadolinium chelate alone. A tendency towards higher signal intensities for Gadofluorine M without Lipofectin can be observed, and in the following experiments the transfection agent was omitted.

Validation of cell labeling after stereotactic implantation

In T1-w MRI of the two animals a signal hyperintensive area in the right hemisphere corresponded to the injected labeled cells, while on the contralateral side an iso-to-hypointense area was present. An inverse signal distribution was found in the T2-weighted (T2-w) images, as the labeled cells induced signal loss in the right hemisphere while an edematous hyperintensive lesion was seen at the implantation site of the unlabeled cells (without Gadofluorine M-Cy3.5) of the left side. The comparison of post mortem histology with the MR images revealed at both implantation sites the BrdU labeled. The amount of erythrocytes due to the trauma of the surgery was comparable for both implantation sites. More over the red fluorescence dye of the Cy3.5 attached to Gadofluorine M was only visible in the right hemisphere (Figure 1).

Discussion

This study describes a novel contrast agent with the capability of intracellular accumulation without an uptake mediator providing a T1-positive MRI signal at 1.5T and enables the discrimination between a hemorrhage and labeled cells.

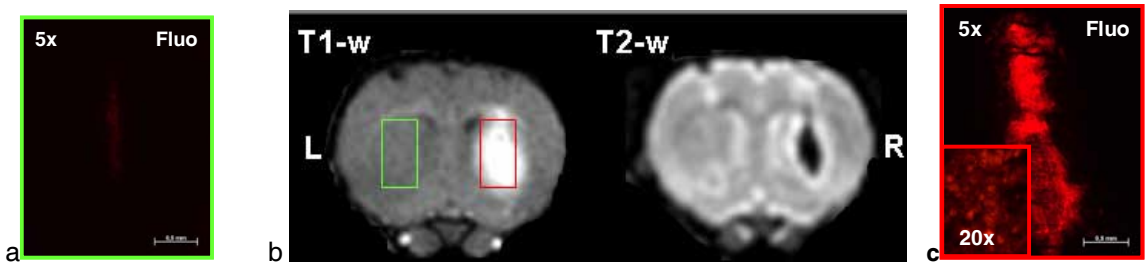

Figure 1: *In vivo* MRI evaluation (b) of the labeling technique after stereotactic implantation of Gadofluorine M labeled MSCs in the right (c, red boxes) hemisphere and unlabeled control MSCs in the left (a, green boxes) hemisphere.