Long term MR signal characteristics of Ferucarbotran-labeled mesenchymal stem cells: Discrimination of intra- and extracellular iron oxides before and after cell lysis

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Introduction.

Promising applications of stem cell based therapy include the replacement of defective tissue in patients with diseases such as diabetes or Parkinson's (1). To evaluate the success of engraftment, it is important to generate information on the viability of the injected cells. Labeling cells for MR imaging with iron oxides provides a means of sensitive cell tracking combined with high anatomical resolution in vivo (2). Since intracellular iron oxides may be metabolized or released, the potential signal changes over time needs to be studied. Therefore, the long term signal effects of viable and dead Ferucarbotran-labeled human mesenchymal stem cells (*h*MSC) at 1.5T and 3T were investigated.

Material and Methods

Ferucarbotran (Resovist®, Schering AG) is a negatively charged iron oxide nanoparticle, approved for clinical use in Europe, with a mean hydrodynamic diameter of 62 nm. The R1 and R2 relaxivities in blood (1.5T, 37°C) are 7.2 ± 0.1 mM $^{-1}s^{-1}$ and 82.0 ± 6.2 mM $^{-1}s^{-1}$, respectively (3). We labeled *h*MSC by simple incubation with Ferucarbotran (100µg Fe/ml) in DMEM media for 24 hours. After labeling, cells were washed, replated and cultured for up to 14 days. Before imaging, cells were counted and cell viability was assessed by trypan blue staining. Two experiments were performed whereby each included different time intervals after the labeling process. In the first experiment, a constant number of cells was evaluated at different dilutions. In the second, cells were evaluated at increasing numbers over time due to cell proliferation. The cells were resuspended in isotonic Ficoll solution at a density of 1.07 g/ml to prevent sedimentation. To compare the signal characteristics of intra- versus extracellular iron oxides, additional, duplicate samples for each time point were prepared and lysed using a sonicator.

MR imaging of the samples was performed using 3T and 1.5T MR clinical scanners (Signa, GE Medical Systems, Milwaukee, WI, USA) and standard circularly polarized quadrature knee coils (Clinical MR Solutions, Brookfield, WI, USA). To avoid susceptibility artifacts from the surrounding air, all probes were placed in a water-containing plastic container at room temperature (20ºC). Axial T1-weighted spinecho (SE)-sequences were obtained with multiple TR values of 4000, 1000, 500, 250 ms as well as a TE of 16 and 15 ms at 1.5T and 3T, respectively. Axial T2-weighted SE-images were obtained with a TR of 4000 ms and increasing TEs of 16, 32, 48 and 64 ms at 1.5T as well as 15, 30, 45 and 60 ms at 3T. Axial T2*-weighted GE-images were obtained with multiple TEs of 3.7/7.2/14.4/28.8 ms, a TR of 500 ms and a flip angle of 30°. All sequences were acquired with a field of view of 160x160 mm, a matrix of 256x196 pixel, a slice thickness of 5 mm and one acquisition. Based on these sequences, T1-, T2- and T2* maps were calculated, assuming a monoexponential signal decay. T1-, T2 and T2*-relaxation times of all samples were determined from these maps. All experiments were performed at least twice. Differences in MR-data between samples of intact and lysed cells were evaluated for significance with a t-test ($p<0.05$). For comparison, the intracellular iron oxide uptake was confirmed with electron microscopy and quantified with ICP-AES of labeled cells and non-labeled controls.

Results

The labeled cells showed a proliferation rate of approximately 5 days. Viability was confirmed by Trypan blue staining. Electron microscopy showed the iron oxide particles in secondary lysosomes of the cells. Samples of constant numbers of hMSC were investigated at different time intervals after the labeling process. ICP-AES showed an initial iron oxide uptake of 7 pg per cell, followed by a slow decline in intracellular iron content over time. Corresponding MR studies showed an initial and marked shortening in T2-relaxation times of labeled cells compared to non-labeled controls. This T2-effect of the labeled cells slowly declined over time. At 12 days after the labeling process, residual T2-shortening of the cell samples and remaining intracellular iron was noted (Fig. 1). The second experiment, involving the proliferated cell population (i.e. increasing cell number over time), showed a nearly constant iron content and stable T1- and T2-data up to 14 days after the labeling process (Fig. 2). The samples with intracellular, compartmentalized iron oxides in viable cells showed a smaller T2-effect as compared to corresponding samples with extracellular, free iron oxides after cell lysis (Fig. 2). This difference between T2 relaxation times of samples with viable and lysed cells was not significantly different at 1.5 and 3T. The significantly higher T2-effect of released iron oxides compared to intracellular iron oxides at identical concentrations may be used to differentiate viable from dead hMSCs.

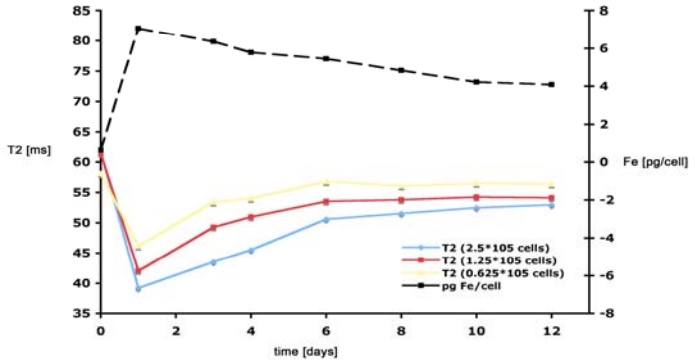

Figure 1: T2 relaxation times of hMSC at different time points after labeling with Ferucarbotran (colored curves) and corresponding intracellular iron content over time as measured by ICP-AES (upper curve). Time = 0 corresponds to cells before labeling.

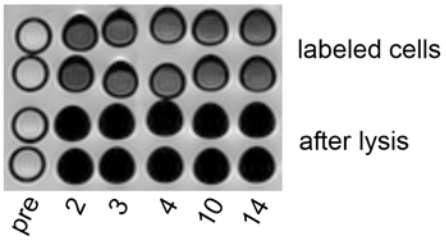

Figure 2: T2-SE 90°/4000/30 image of labeled cells at different time points before and after lysis, corresponding to intracellular and extracellular iron. Note the obvious difference in signal intensity between intra- and extracellular iron, which can be used to discriminate viable from dead cells. However, there is no significant signal decay over time.

Conclusion

Viable Ferucarbotran-labeled *h*MSC and released Ferucarbotran after cell lysis can be differentiated by significant differences in R2-data. This can be used to differentiate viable from non-viable hMSCs. The contrast agent content per cell decreases over time. Within a 14-day period of observation, the effect is mainly due to the distribution of the iron oxides in proliferating progenies and not metabolization, since the iron content in the whole cell population remains nearly unchanged. The described effect on T2 relaxation times was not significantly different for 1.5 and 3T.

References

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