Comparison of labeling strategies for stem cells with Gd-chelates.

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INTRODUCTION

Stem cell labelling for MRI-based *in vivo* monitoring of cell-migration is usually performed using iron oxide particles [1, 2]. Lanthanide chelates have been used in only a few feasibility studies for the detection of cell deposits [3, 4]. Sensitivity limitations of lanthanide chelate labelling in T1-weighted MR images were thought to be overcome by increased loading of the cells with the contrast agent. However, Aime et al. indicated that loading by endocytosis can be saturated [3]. It was the aim of this study to test commercially available Gd-chelates for labelling different embryonic stem cells and neural progenitor cell-lines and their visualisation *in vitro* and *in vivo*. An assessment of different labelling strategies was performed.

METHODS

Cell labelling: The murine embryonic stem cell-lines D3 and L1 as well as the neural progenitor cell-line C17.2 were labelled with commercially available Gd-chelates (Magnevist® and Prohance®) by endocytosis or electroporation (U=240V, t=10-20 ms). Uptake by endocytosis was tested after incubation for up to 48h with up to 100mM Gd-chelate in the medium. Cells were washed four times before *in vitro* testing or implantation into rat brains.

Toxicity and proliferation were tested in cell suspensions. Visualisation limits were determined in phantoms and after stereotactic implantation in the brain of Wistar rats.

Animal model: All experiments were performed in accordance with the NIH animal protection guidelines and approved by the local governmental authorities. 10,000-1,000,000 cells suspended in 2 μl were stereotactically implanted in Wistar rats (0.5 mm anterior, 3.0 mm lateral to bregma, 2.0 mm ventral from the dural surface). Animals were imaged immediately after implantation and 2 to 10 days thereafter.

MRI: T1-weighted MR images were acquired using a Bruker Biospin 7.0 Tesla small animal scanner equipped with an actively shielded gradient set of 200 mT m^{-1}. For rf irradiation and signal detection custom-built coils were used. A 5-cm-diameter transmit-receive coil was used for agar phantoms and a 12-cm-diameter Helmholtz coil arrangement served for rf excitation and a 3.0 cm diameter surface coil for signal detection in animal models. T1-weighted 3D MR images were acquired using gradient echoes (FLASH) with TR=100ms, TE=5ms, 70º pulse. The isotropic spatial resolution was 78 μm for phantoms and for animal experiments. MR images were processed with the NIH software 'Image J'.

RESULTS & DISCUSSION

Incubation of embryonic stem cells (D3 and L1) with Gd-chelates did not result in efficient uptake for *in vitro* detection in phantoms or *in vivo* in animal models. Electroporation of cell suspensions containing up to 100mM Gd resulted in stable labelling of all cell-lines. Minimum detectable cell numbers were approximately 5,000 cells μl^{-1} in phantoms (see Fig. 1) and 20,000 μl^{-1} in animal models. Cells were detectable for up to five proliferation cycles after labelling. The Gd-chelates did not show adverse effects on cell viability and proliferation for concentrations < 20mM.

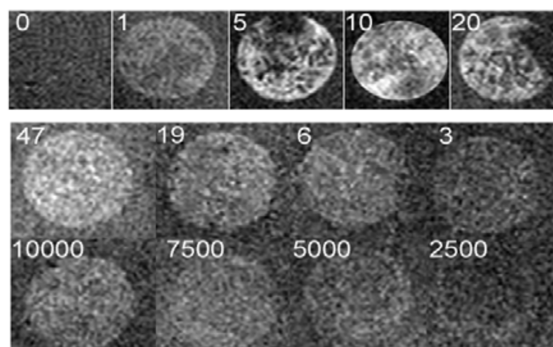

Fig.1: T1w MRI of agar phantoms loaded with Gd-chelate labelled C17.2 cells.
First row: Endocytosis of Gd-chelate (concentrations indicated in mM) by incubation with C17 cells. Each circular volume was loaded with 30,000 cells μl^{-1}.
Second row: Electroporation using indicated Gd-chelate concentrations (mM). Each volume was loaded with 10,000 cells μl^{-1}.
Third row: Electroporation using 47mM Gd-chelate in the medium. Each volume contained the indicated cell density in cells μl^{-1}.

As indicated in Figure 1, the R1 relaxivity increased with increasing intracellular Gd-chelate concentrations when cells were labelled by electroporation. However, endocytosis of Gd-chelates by C17.2 cells, resulting in the same intracellular concentration as for electroporation, showed saturation. This might be due to a lack of water in the endosomes as suggested by Aime et al. [3].

CONCLUSIONS

Although less sensitive than iron oxide particles, Gd-chelates are suitable for the visualization of stem cells *in vivo*. The route of internalization is an important factor for efficient labeling.

REFERENCES

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