A responsive MRI contrast agent to monitor functional cell status: a feasibility study using dendritic cells.

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INTRODUCTION

Molecular Imaging of transplanted stem and progenitor cells is essential to understand their therapeutic potential. Stable labeling of cells with either iron oxide particles (USPIO) or lanthanide chelates has proven successful for MRI-based detection of cell deposits and their migration [1, 2]. However, MRI only provides information about the location but not about cellular change of the functional status (e.g. differentiation). This information can be provided by responsive lanthanide chelate-based contrast agents that only generate T1-contrast after enzymatic activation. Lanthanide-based probes responsive to β-galactosidase resulted in an increase of the R_1 relaxivity by 20% up to three times the R_1 in the "inactive" state [3, 4]. Although applicable for *in vivo* applications [4], the remaining relaxivity in the inactive state due to the secondary coordination sphere makes the probe too insensitive for *in vivo* cell tracking. The aim of this study was to develop and test a Gd-chelate based contrast agent for conditional activation by intracellular lipase with zero T1 relaxivity in its inactive state in a proof-of-concept study.

METHODS

Contrast agent: The Gd-DTPA chelate was linked with two long fatty acid chains ($C_{17}H_{35}$) through ester bonds (Figure 1). The insoluble Gd-DTPA-FA complex had a relaxivity of zero and the activated complex an *in situ* relaxivity of 4.7 mM^{-1} s^{-1}. Micronization resulted in particle sizes of 0.5 to 1 μm.

Cell labelling: Flt3+CD11b+progenitor cells were isolated from mice according to [5]. Undifferentiated cells and fully differentiated dendritic cells were incubated with the Gd-DTPA-FA complex (1 to 20mM) for up to 24 hours, washed four times and either used for *in vitro* testing or implanted in the brain of Wistar rats (n=4).

MRI: T1-weighted MR images were acquired using a Bruker Biospin 7.0 Tesla small animal scanner equipped with an actively shielded gradient sets of 200 mT m^{-1} using 3D gradient echo sequences (FLASH) with TR=120ms, TE=5ms, 70º pulse, FOV= 3x3x1cm (animal model) and 4.5x4.5x1cm (agar phantoms), the isotropic spatial resolution was 78 μm for phantoms and 50 μm for animal experiments. 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 with a 3.0 cm diameter surface coil for signal detection for animals.

Animal model: 10,000-1,000,000 cells suspended in 2 μl were implanted in normal Wistar rats (n=4) into the border between the cortex and the corpus callosum (0.5 mm anterior, 3.0 mm lateral to bregma, 2.0 mm ventral from the dural surface) using sterotactic injection. Animals were imaged immediately after implantation and 4-14 days thereafter.

RESULTS

The intracellular activation of the insoluble Gd-DTPA-FA complex was shown for dendritic cells and to a lesser extent (20% of the relaxivity achieved in dendritic cells) for the progenitor cells. The T1 relaxivity increased to a saturation value of 3.9±0.5 mM^{-1} s^{-1} (n=4) within nine hours of incubation (20mM contrast agent in the medium). The supernatant (medium) showed a relaxivity of only 0.5±0.3 mM^{-1} s^{-1}, indicating that the Gd-DTPA-FA complex was only activated intracellularly. The minimum detectable concentration *in vitro* was 20,000 cells (signal intensity fourfold compared to unlabelled controls).
In vivo detection limits were 50,000 cells, which were detectable for up to 7 days after implantation of cells labeled with 20mM Gd-DTPA-FA. 200,000 cells were also detectable after 14 days.

CONCLUSION

We were able to show that insoluble Gd-chelates are a suitable contrast agent for conditional activation by intracellular lipases. The chelate can easily be modified to be targeted by enzymes expressed during specific change of cell status. Such a system will then be suitable for functional cellular MR imaging.

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