Polyamine-substituted Gd-DTPA: intracellular, tumor-selective, high-retention MRI contrast agents

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Introduction

Conventional paramagnetic MRI contrast agents such as $[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist®) exhibit low tissue specificity. Therefore, increasing efforts are being made to develop targeted agents [1] through conjugation with monoclonal antibodies [2] or modification to achieve high affinity for a tumor-specific cell-surface protein, for example [3]. An alternative strategy is to employ *intracellular* uptake to "label" the cells of interest. This requires, for detectable T_1-weighted MRI contrast, internalization of 10^7 - 10^8 Gd(III) complexes (0.017 - 0.17 fmol) per cell. However, clinical contrast agents do not readily enter cells passively or via active transport. It has been shown that ligand sidechains containing alkylamine or polyamine moieties enhance the intracellular uptake of technetium complexes or radioiodinated benzamides for melanoma scintigraphy [4,5], presumably by active transport via polyamine transporter proteins. Since proliferating tumor cells are expected to have enhanced polyamine uptake, our strategy was to develop a new class of polyamine-derivatized Gd-DTPA complexes with high *intracellular* tumor uptake and retention.

Methods

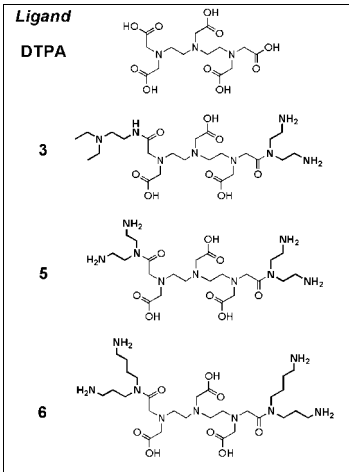
Symmetric and asymmetric bis(amide) ligands such as **3**, **5**, and **6** (Scheme 1) were synthesized by straightforward aminolysis of DTPA-dianhydride. Addition of Gd(III) resulted in the chelation complexes **Gd-3**, **Gd-5**, and **Gd-6**. Uptake studies were performed in cell culture with B16 mouse melanoma and MH3924A Morris hepatoma (rat) cells. Following 24-h incubation with 0 - 10 µM Gd complex (triplicate samples), cells were harvested (ca. 3×10^6) and digested in 50% HNO_3. Gd content of the digests was determined by element mass spectrometry (ICP-MS) using rhodium as internal standard. Control experiments were performed with the extracellular agents Magnevist® and Gadovist®. In vivo imaging studies were performed at 2.35 T (Bruker BioSpec 24/40) with ACI rats bearing a subcutaneous MH3924A tumor in the right thigh. Following an intravenous bolus of contrast agent (0.1 mmol/kg), T_2-weighted (multi-spin-echo, TR = 2 s, TE = n×8 ms) and T_1-weighted (gradient-echo, TR/TE = 212/5 ms, 60° flip) multislice data sets were acquired with FOV = 70×70 mm and 2-mm slices (128×128 pixels).

Results

The three Gd complexes whose polyamine-substituted DTPA ligands are shown in Scheme 1 exhibited molar relaxivities r_1 of 2 - 8 s^{-1} mM^{-1} at 5.9 T and 37°C (r_1 = 4 for Gd-DTPA). Fig. 1 shows that cellular uptake for both tumor cell types was proportional to complex concentration and increased in the order **Gd-5** < **Gd-6** < **Gd-3**. At 10 µM incubations, uptake in the range 0.02 - 0.2 fmol/cell was achieved, corresponding to intracellular concentrations of ca. 11 - 110 µM. Incubations with Gd complex for 1 h followed by a 24-h washout period showed that there was high retention of intracellular contrast agent. Uptake of Magnevist® was below the detection limit of ICP-MS under all incubation conditions. The following evidence argues for facilitated uptake of the new complexes via polyamine transporters: (a) uptake was nearly abolished at 4°C (1-h incubations) and (b) uptake was effectively inhibited by 20 µM benzyl viologen, a potent inhibitor of polyamine transport. The complexes showed no significant binding to serum proteins and no toxicity to tumor cells at 100 µM for 48 h. The results of Fig. 2 illustrate the utility of **Gd-6** vs. Gadovist® as a tumor-specific contrast agent in vivo. In the top images T_2 weighting provides good delineation of tumor as a heterogeneous hyperintense region, independent of contrast agent. The middle images show that at 1 h **Gd-6** provides much higher T_1-wt. contrast for tumor compared to Gadovist® and that contrast enhancement with **Gd-6** is still evident at 24 h post Gd (bottom). Analogous results were obtained with **Gd-3** and **Gd-5**.

Conclusions

Gd(III) contrast agents with polyamine-substituted DTPA ligands have multiple positive charges, exhibit high uptake by facilitated transport into two different tumor cell types, and are retained within tumor cells for at least 24 h. In vivo studies with a rat model demonstrate that the expected T_1-wt. contrast enhancement in tumor is achieved with the novel agents, even at 24 h post Gd injection.



Scheme 1. Polyamine-substituted DTPA ligands.

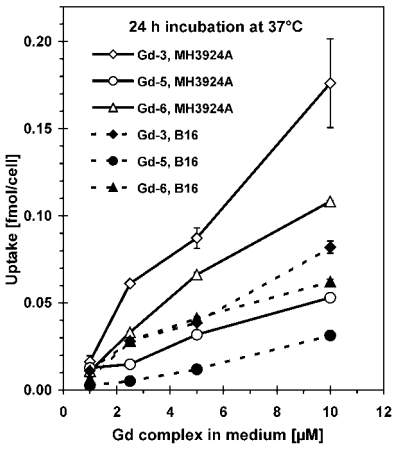


Fig. 1. Cellular uptake of contrast agents in cell culture.

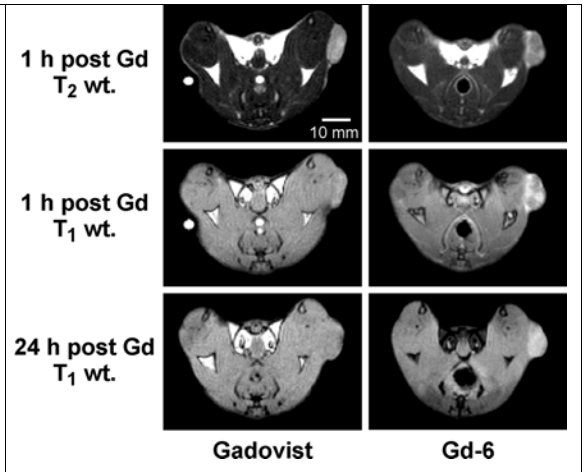


Fig. 2. MRI at 2.35 T with rat tumor model following 0.1 mmol/kg contrast agent.

References

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