Silver Nanoparticles Functionalized with High Gadolinium Chelate Payload As Effective In Vivo T1-Brightening Contrast Agents

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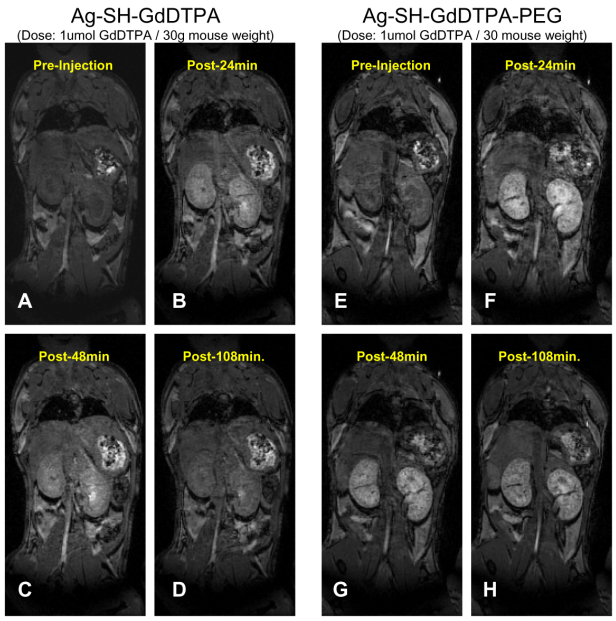
Introduction: The twin aims of designing MRI multimodal agents have been to narrow the bio-distribution of the agent and to amplify its image enhancement. The growth and development of nanoparticle technology is key in the formation of such multimodal agents that promise large image enhancement by carrying a multiplicity of interior or surface bound contrast agents [1-2]. Gold or silver metal nanoparticles offer attractive multivalent and multimodal platforms for imaging with their ability to bind a cocktail of molecules that are attached to the particle surface via thiol terminated linkers. Our group [3] and others [4-8] have demonstrated that this approach to MRI enhancement can provide a very effective T1-contrast multiplicative effect through a high Gd payload. Although surface derivatization of noble metal nanoparticles is readily achieved, the resulting particles have properties that are determined by charge and steric interactions between the surface entities. In some cases, surface modifications can render a particle insoluble in aqueous media, or make it prone to aggregation in salt solutions such as phosphate buffer. In the research described here we designed a new silver multimodal nanoparticle containing GdDTPA and tested the effect of coligated polyethyleneglycol (PEG) chains on particle distribution, clearance and contrast enhancement. The resulting GdDTPA content, relaxivity and solubility were characterized in vitro and their biodistribution was assessed by abdominal MRI.



Material and Methods: Synthesis: Silver nanoparticles were prepared by the method of Kim et al. [9] and were derivatized using a chemically modified DTPA with a thiolate terminated tether at the β carbon of the DTPA central carboxylate group. The bifunctional ligand is anchored to silver via the thiol and binds gadolinium with five carboxylate and three secondary amine groups. MRI: In vitro and In vivo testing of this nanoparticle was performed on a 7-T Bruker micro-MRI system, interfaced to a 200-mm horizontal bore magnet (Magnex Scientific, Yarnton, UK) with 750-mT/m an actively shielded. To monitor the effect of the nanoparticles over a 2-hour period, a modified 3DGE sequence was used which acquires a self-gating signal on the readout dephasing gradient within each TR [10]. The gating signal was used retrospectively for artifact-free image reconstruction. The *in vivo* protocol in C57Black6 wild type mice consisted of a pre-injection scan to acquire a contrast agent-free datasets followed by seven consecutive post-femoral injection scans to monitor the MRI contrast and the biodistribution throughout the abdomen (see Fig. 1) of either GdDTPA (Magnevist, 3µmol/30g mouse body weight, data not shown), Ag-SH-GdDTPA (1µmol equivalent Gd/30g) or Ag-SH-GdDTPA-PEG (1µmol equivalent Gd/30g) with a total experiment duration for each individual mouse of less than 3-hours including the calibration step. The parameters of the sequence were as follows: FOV = 51.2x25.6x25.6 mm^3, Matrix= 256x128x128, resolution=$(200\mu m)^3$, TR/TE=15/4 ms, Averages=3, acquisition time=12-min. The Flip Angle (18º) was chosen to provide the greatest T1-enhancement contrast [8]. During the MR image acquisition, mice were anesthetized with Isoflurane and the body temperature was maintained at 35/37oC using a heating water bed.

Results and Discussion:

All the image sets shown above were obtained using a coronal slice orientation. Figures A,B,C,&D correspond to a set of images obtained from a time course study of mouse subjected to an injection of Ag-SH-GdDTPA. In contrast to mice injected with Gd-DTPA injection (3umol/30g, single clinical dose, data now shown), the enhancement observed with our nanoparticles was notably more pronounced over the course of our study particularly in the kidneys beyond the clearance observed for magnevist. Surprisingly our 15-nm nanoparticles demonstrated enhancement in the kidneys within minutes using a minutes temporal resolution after injection (data not shown) suggesting renal clearance. Furthermore the incorporation of PEG in the nanoparticles further increased both the enhancement and the longevity of our compound in a time course study illustrated in the example of Figure E-H. Our preliminary studies both *in vitro* (data not shown) and *in vivo* study clearly demonstrates a improvement of both the relaxivity and solubility of our silver nanoparticles compared to the non PEG version and to clinical Magnevist.

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