A Targeted Nanoglobular Manganese(II) Chelate Conjugate for Magnetic Resonance Cancer Molecular Imaging

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Introduction:

Mn(II) chelates are attractive as potential MRI contrast agents because Mn(II) has relatively high electronic spins (5/2) and fast water exchange rates[1]. Small molecular Mn(II)-based MRI contrast agents have been investigated over the past decades. Two agents, the hepatocyte-specific Mn(II) dipyridoxal diphosphate (Mn-DPDP) and an oral agent containing $MnCl_2$ (LumenHance) for gastro-intestinal imaging, have been clinically used for human[2]. However, it remains relatively less studied compared to Gd(III)-based MRI contrast agents. One of the limitations of these contrast agents is their low specificity. Dendrimers are a unique class of polymers with well-defined structures and nanosizes. The large number of functional groups at their periphery can be used for conjugating contrast agents and targeting moieties to develop targeted MRI contrast agents with improved sensitivity and specificity. It is reported that cyclic CLT1 peptide can specifically bind to fibrin-fibronectin complexes formed by clotted plasma protein and show effective tumor targeting[3]. The fibrin-fibronectin complexes may be a suitable molecular target for cancer molecular imaging with MRI. Here, we report a CLT1-targeted dendrimeric Mn(II)-DOTA conjugate as a targeted contrast agent for cancer MR molecular imaging.

Methods:

Targeted contrast agent

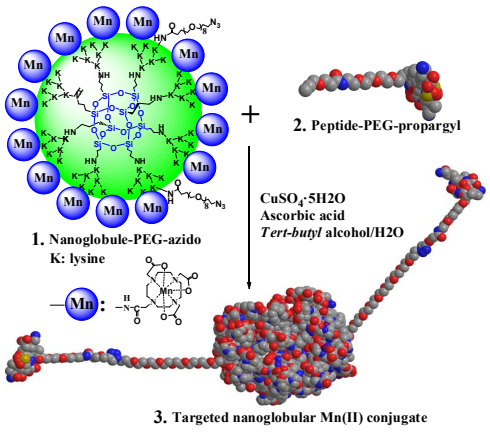
Nanoglobules, lysine dendrimers with a silsesquioxane core, were synthesized in good yield and purity using solution phase peptide chemistry [4]. CLT1 Peptide was synthesized by solid phase peptide chemistry and conjugated to a nanoglobular contrast agent via click chemistry. A non-targeted contrast agent was also prepared for comparison. Characterization and structure confirmation of intermediates and products were carried out by HPLC, MALDI-TOF mass spectrometry and amino acid analysis. Gd content in the contrast agent was determined by ICP-optical emission spectroscopy.

In vivo MR imaging

Contrast enhanced MRI was performed in female nu/nu athymic mice bearing MDA-MB-231 human breast carcinoma xenografts. All MRI experiments were performed on a 3 Tesla Siemens MRI scanner. The peptide targeted and non-targeted nanoglobular contrast agents were administered via a tail vein at a dose of 0.03 mmol-Gd/kg. Tumor contrast enhancement was measured and expressed as contrast-to-noise ratios (CNR).

Results and discussion:

A CLT1 peptide-targeted G3 nanoglobular contrast agent, Mn-(DOTA-monoamide) conjugate of poly-$_L$-lysine dendrimer with a cubic octa(3-amino-proppyl)silsesquioxane (OAS) core was prepared as shown in Scheme 1. Approximately 2 peptides and 42 Mn chelates were conjugated to the surface of 64 amine groups of G3 nanoglobules. The r_1 and r_2 of the targeted agent were 3.13 and 8.74 $mM^{-1}s^{-1}$ per Mn(II) chelate at 3T. Significant tumor enhancement was observed in the tumor after the injection of the targeted agent, while little enhancement was observed in the tumor with the control agent (Figure 1). Quantitative analysis of the CNR in the tumor tissue revealed that CTL1-targeted nanoglobular contrast agent resulted in a significant increase of CNR within tumor as compared to the non-targeted control ($p < 0.05$ except time point t =15 min). The data demonstrated that the targeted agent could deliver sufficient amount of the Mn(II) chelates to tumor by specific binding, resulting in significant tumor enhancement.



Scheme1. Synthetic scheme of a CLT1-targeted nanoglobular Mn(II)-DOTA conjugate via click chemistry.

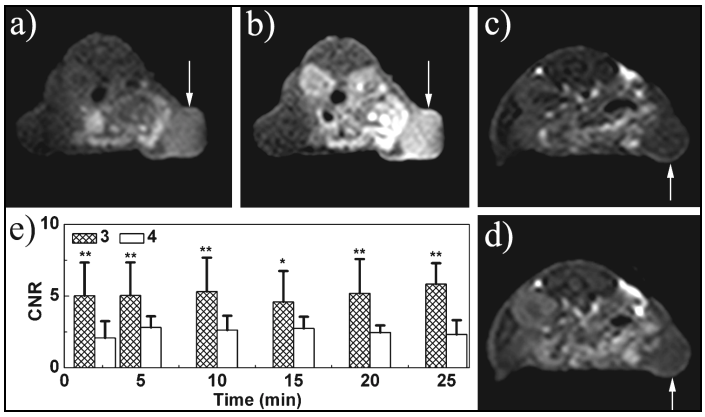


Figure 1. 2D axial MR images of tumor xenografts before (a) and 25 min after (b) the administration of targeted Mn(II) agent and before (c) and 25 min after (d) the administration of non-targeted Mn(II) agent. Arrows point tumors. (e) CNR in the tumor with targeted and non-targeted Mn(II) agent administrated at 0.03 mmol-Mn/kg in the tumor bearing mice. **p < 0.05, *p <0.06.

Conclusions:

A peptide-targeted nanoglobular Mn(II)-DOTA conjugate was designed and synthesized for cancer MR molecular imaging. The TLC1-targeted contrast agent resulted in significant enhancement in tumor at a relatively low dose as compared to the non-targeted control. Further studies are needed to optimize the structure of the Mn(II) based targeted contrast agents and to demonstrate their safety for further development. The CLT1-targeted nanoglobular Mn-DOTA conjugate is promising for cancer molecular imaging with MRI.

References:

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