A CLT1 peptide targeted nanoglobular contrast agent for cancer molecular imaging with MRI

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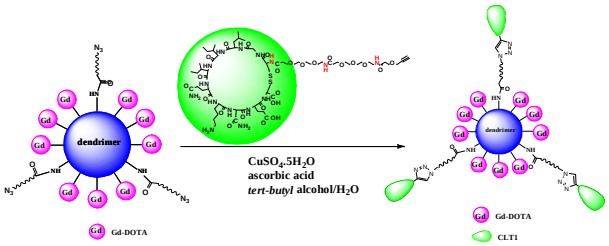
Introduction:

Dendrimer-based MRI contrast agents (CAs) have received considerable attention because these macromolecular agents provide strong and prolonged enhancement at reduced doses. The highly functionalized surface of dendrimers enables these uniform macromolecules to become an excellent platform for multimodal imaging agents and targeted imaging agents. However, the flexible structure of conventional dendrimeric carriers may affect the pharmacokinetic properties and targeting efficiency of dendrimeric imaging agents. A macromolecular contrast agent with a compact globular structure would minimize the nonspecific tissue interactions and improve targeting efficiency, which can reduce background noise and enhance selectivity for molecular imaging. Cyclic CLT1 peptide can specifically bind to fibrin-fibronectin complexes formed by clotted plasma protein and has shown effective tumor targeting[1]. We have designed, synthesized and evaluated a CLT1 peptide-targeted globular dendrimeric contrast agent with precisely defined nanosizes as a targeted nanoglobular MRI contrast agent, for cancer molecular imaging with MRI.

Methods:

Nanoglobules, lysine dendrimers with a silsesquioxane core, were synthesized in good yield and purity using solution phase peptide chemistry[2]. CLT1 Peptide was synthesized by solid phase peptide chemistry and conjugated to a nanoglobular contrast agent via click chemistry. 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. Contrast enhanced MRI was performed in female nu/nu athymic mice bearing MDA-MB-231 human breast carcinoma xenografts. 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 enhancement ratios, which were calculated as the ratio of the signal in tumor to that of the thigh muscle.



Scheme 1. Synthesis of peptide targeted G2 nanoglobular contrast agents

Results:

A CLT1 peptide-targeted G2 nanoglobular contrast agent, Gd-(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 3 peptides and 25 Gd-DOTA chelates were conjugated to the surface of 32 amine groups of G2 nanoglobules. The T_1 relaxivity (r_1) at room temperature is 7.2 mM^{-1} sec^{-1} at 3T. The contrast enhanced MRI in tumor bearing mice showed that the peptide targeted and non-targeted nanoglobular contrast agents had relatively long blood circulation and cleared via renal filtration. The CLT1-peptide targeted nanoglobular contrast agent showed much higher enhancement than the non-targeted nanoglobular contrast agent in the tumor, Figure 1. Figure 2 shows the axial 2D spin echo images of tumor tissues for peptide targeted and non-targeted contrast agents at a dose of 0.03 mmol-Gd/kg. The targeted contrast agent resulted in more significant tumor enhancement than the non-targeted agent.

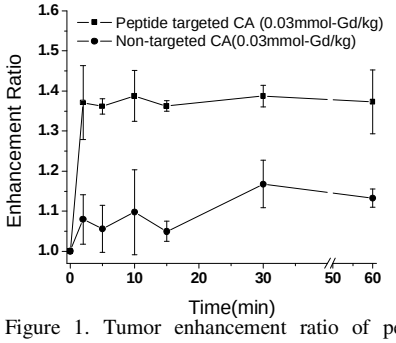


Figure 1. Tumor enhancement ratio of peptide targeted and non-targeted nanoglobular CAs

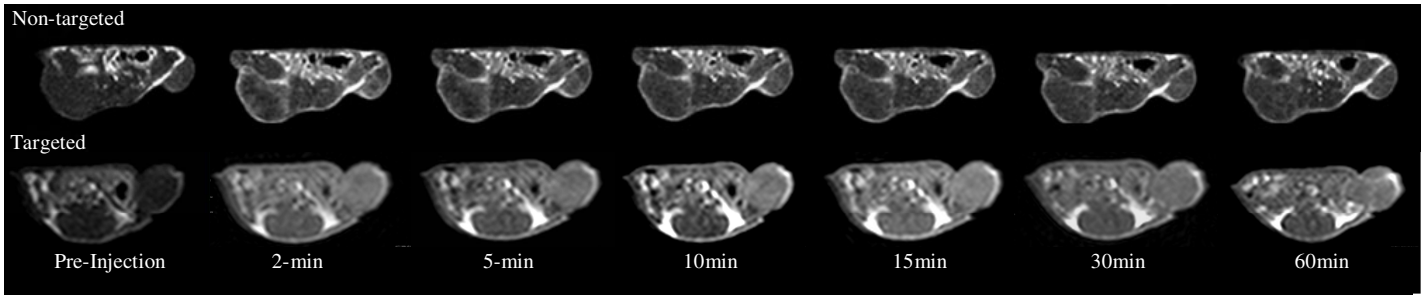


Figure 2. 2D axial images of tumor xenografts of Cyclic-CLT1 peptide targeted nanoglobular MRI CAs administered at 0.03 mmol Gd/kg in nu/nu female nude mice.

Conclusions:

A CLT1 peptide targeted G2 nanoglobular MRI contrast agent was synthesized by click chemistry. The targeted contrast agent resulted in substantial contrast enhancement in the tumor. The targeted contrast agent has a potential for specific cancer molecular imaging with MRI.

References:

[1] J. Pilch, D.M. Brown, M. Komatsu, et al. *Proc. Natl. Acad. Sci. USA* 2006, *103*, 2800–2804.

[2] T. Kaneshiro, X. Wang, Z. Lu. *Mol. Pharm*. 2007, *4*, 759-768;