Towards Target-Specific Molecular Imaging of Angiogenesis with Gd-DTPA-Based Dendritic Architectures

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INTRODUCTION

Molecular imaging is a promising modality for the visualization of local cellular and physiologic processes, such as angiogenesis, i.e. new blood vessel formation. On a cellular level the expression of angiogenesis-specific proteins may be targeted with the use of target specific MRI contrast agents modified with oligopeptide residues. On a local tissue level MRI contrast agents have been shown to be effective in reflecting physiologic processes in tumor angiogenesis. Current clinically available gadolinium-based contrast agents are sub optimal for molecular imaging for the reasons that such agents are non-tissue specific and have relatively low relaxivities. Dendritic MRI contrast agents are attractive candidates for molecular imaging of tumor angiogenesis, since the multivalent dendritic architecture is an ideal molecular scaffold for constructing multiple MRI labels and target specific markers to a single molecule. Recently, we reported on the synthesis of gadolinium-diethylene-triaminepentaacetic acid (Gd-DTPA)-functionalized poly(propylene imine) dendrimers (figure 1).

Figure 1. Schematic representation of Gd-DTPA-functionalized poly(propylene imine) dendrimers. 7,8

Higher generations of these dendritic MRI contrast agents display a pronounced enhancement of both the longitudinal and the transverse relaxivity compared to clinically available Gd-DTPA (Magnevist®, Schering, Germany). The efficiency of our dendritic MRI contrast agents might further be improved by the incorporation of target-specific oligopeptides for tumor angiogenesis. For instance, cyclic NGR is anticipated to bind preferentially to the tumor vasculature. Here, we report on the kinetic physiologic properties of different generations of the Gd-DTPA-functionalized dendritic contrast agents and the synthesis of Gd-DTPA complexes functionalized with a cyclic NGR motif.

RESULTS AND DISCUSSION

Gd-DTPA-functionalized dendritic MRI contrast agents in vivo

Dynamic Contrast Enhanced MR Angiography (DCE-MRA) experiments were performed in mice using a surface coil (5 cm diameter) at 1.5 T with the first (G1) and fifth (G5) generation of the dendritic contrast agent, containing respectively 4 and 64 Gd-DTPA units per molecules. The contrast agent was administrated intravenously in the tail vein at a dose of 0.03 mmol Gd/kg body weight. MR images were obtained immediately after administration (8 s), at 30 s and 8 min. The first generation dendritic contrast agent (3.0 kDa) is cleared from the renal system and accumulates rapidly in the bladder (Figure 2).

Figure 2. DCE-MRA experiments with 0.03 mmol Gd/kg body weight of G1 in mice at 1.5 T.

The fifth generation dendritic contrast agent (51.0 kDa, diameter 5-6 nm) is cleared from the renal system at a slower rate than the first generation dendritic contrast agent. Transient accumulation in the hepatic and splenetic system was observed. The renal excretion of the fifth generation dendritic contrast agents and its accumulation in the bladder supports that this MRI contrast agent is small enough to pass through the slit pores of the glomerular membrane in the kidney. Timely clearance through the kidney is important for Gd-DTPA-based MRI contrast agents to prevent the undesired accumulation of free Gd(III) in the body.

Synthesis of cyclic NGR-Gd-DTPA

A solid phase peptide synthesis (SPPS) strategy is employed to prepare Gd-DTPA-functionalized oligopeptide containing the target specific cyclic NGR sequence. Manual SPPS using the in situ neutralization/2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HBTU) activation procedure for tBoc chemistry on MBHA resin was applied to synthesize the peptide AcCNGRCGGK(Fmoc)-MBHA containing the target-specific asparagine-glycine-arginine (NGR) sequence. Subsequently, on the resin, the Fmoc from the lysine side-chain was removed and...
Ac-CNGRCGGK(NH₂)-MBHA was reacted with an isocyanate-functionalized DTPA synthon to yield Ac-CNGRCGGK(DTPA)-MBHA. Cleavage of the Ac-CNGRCGGK(DTPA) from the resin with HF followed by purification using reversed-phase HPLC afforded the peptide labeled with DTPA as shown in Scheme 1.

Scheme 1. SPPS of Ac-CNGRCGGK-DTPA, reacting Ac-CNGRCGGK(NH₂)-MBHA with the isocyanate-functionalized DTPA synthon and cleavage from the resin with HF (R = fBu; R' – R'''' = protecting groups of the different amino acids).

Formation of the disulfide bridge in 1M of guanidine hydrochloride in 0.1 M Tris buffer at pH 8, followed by reversed phase HPLC and lyophilization, yielded cyclic NGR-DTPA (Scheme 2). The corresponding compound was characterized with HPLC and ES-QTOF-MS ([M+2H]²⁺ = 662.4 amu). The corresponding gadolinium complex was prepared by adding 0.9 equivalents of gadolinium chloride to a solution of cyclic NGR-DTPA in water, while the pH was maintained at 7 by adding ammonium hydroxide. A slight excess of cyclic NGR-DTPA was used to ensure the absence of unbound gadolinium. The corresponding gadolinium complex was characterized with IR-spectroscopy and ES-QTOF-MS, while the gadolinium content was determined by means of inductively coupled plasma (ICP).

Scheme 2. Synthesis of cyclic NGR-Gd-DTPA by oxidative formation of the disulfide bridge and subsequent complexation with gadolinium chloride.

Longitudinal (r₁) and transverse (r₂) relaxivities were determined by measuring the concentration dependency of the relaxation times at 1.5 T and 20 °C. The data gave a good linear fit (R² > 0.999) to the equation \(1/T_1 = (1/T_1)_{\text{diamag}} + r_1 \text{[Gd]}\), and a \(r_1\) of 9.8 mM⁻¹s⁻¹ and a \(r_2\) of 11.7 mM⁻¹s⁻¹ were calculated.

Figure 4. Longitudinal (left) and transverse relaxivities (right) of cyclic NGR-Gd-DTPA.

In the near future, this oligopeptide functionalized MRI contrast agent will be tested for MR imaging of angiogenesis in vivo. Moreover, conjugation of both target-specific peptides and MRI contrast agents on dendritic molecules may lead to an even larger improvement, both in binding as a result of multivalency and in terms of signal amplification.

CONCLUSIONS

Dynamic contrast enhanced MR angiography experiments with Gd-DTPA-functionalized poly(propylene imine) dendrimers in mice showed that the first generation of the dendritic contrast agent was rapidly cleared by the renal system, while the fifth generation dendritic contrast agent was cleared at a significantly slower rate. A MRI contrast agent was prepared composed of Gd-DTPA and cyclic NGR, which will be tested in vivo for MR imaging of angiogenesis.

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