RGD Targeted Poly(L-Glutamic Acid)-Cystamine-(Gd-DOTA) Conjugate for Detecting an Angiogenesis Biomarker αvβ3 Integrin with MR T1 mapping

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Introduction. Specific and effective imaging of angiogenesis has a great potential for more accurate detection of angiogenic diseases and assessment of the effectiveness of antiangiogenic therapies. The αvβ3 integrin is one of the biomarkers of angiogenesis1-3. RGD is a peptide motif that can specifically bind to the αvβ3 integrin. We report here an Arg-Gly-Asp (RGD) containing poly(L-glutamic acid)-cystamine-(Gd-DOTA) conjugate for detection of the αvβ3 integrin in tumor tissues with quantitative MR T1 mapping.

Materials and Methods. The peptide c(RGDfK) containing poly(L-glutamic acid) (PGA)-(Gd-DOTA) was synthesized stepwise according to the following scheme. Poly(L-glutamic acid)-(Gd-DOTA) was also synthesized as a control using similar synthetic approach.


The contents of peptide and Gd were determined by H1-NMR, amino acid analysis, and ICP-AES. The binding affinity of the RGD containing conjugate was studied by the vitronectin assay, a αvβ3 integrin dependent adhesion assay, with the DU145 and SLK cancer cells according to the literature4. The αvβ3 integrin is expressed at the cellular surface of both DU-145 and SLK cells. The efficacy of the targeted conjugate for detecting the αvβ3 integrin was evaluated in male nude nude mice (25-30g) bearing human prostate carcinoma DU145 and Kaposi’s sarcoma SLK xenografts in the flanks. The MRI data were acquired on a Siemens 3T Trio MRI scanner. A spoiled gradient-echo pulse sequence with multiple flip angles (128x64 imaging matrix, 300 ms TR, 1.75 ms TE, and flip angles 10, 20, 30, and 45°) was used for T1 mapping. The spatial resolution was 1.0x1.0x2.0 mm3. A 3D-FLASH pulse sequence (2.74ms TE, 7.75 ms TR, 25° flip angle, 120 mm FOV) was applied immediately after the T1 mapping. T1 maps and MR images were acquired before and at various time points up to 20 minutes after the injection of the conjugates at a dose of 5 µmol-Gd/kg. The data were processed by a homemade software using IDL (Interactive Data Language, Boulder, CO).

Results and Discussion. The content of c(RGDfK) is approximately 13 molar-%, and gadolinium content is 27 molar-%. The gadolinium content in the non-targeted control conjugate was 21 molar-%. T1 relaxivity was 9.7 and 8.2 mM⁻¹s⁻¹ per complexed Gd(III) ion for the RGD targeted conjugate and the non-targeted conjugate, respectively. In vitro competitive cell adhesion assay indicate that the RGD containing PGA-cystamine-(Gd-DOTA) conjugate had slightly slow binding affinity to the αvβ3 integrin at the surface of both DU145 and SLK cells as compared to the cyclic peptide c(RGDfK), possible due to the steric effect of the polymer chains. The targeted conjugate has a higher binding affinity to the DU145 cells than the SLK cells. The non-targeted conjugate showed no effect on cellular adherence (Figure 1).

Figure 1. Percentage of cell attachment to the vitronectin-coated plates inhibited by an EMEM serum free medium (control) or solutions of c(RGDfK) (RGD), RGD targeted PGA-cystamine-(Gd-DOTA) and PGA-cystamine-(Gd-DOTA) (P) at the concentrations of 5 µM (closed bar) and 50 µM (open bar).

Figure 2 shows coronal T1-weighted MR images and color-coded T1 maps before and after the injection of contrast agents. A very low dose was used to minimize the non-specific accumulation of the contrast agents. No significant contrast enhancement was observed in the tumor tissues in the T1-weighted images with both targeted and non-targeted conjugates as compared to the precontrast images. The tumor tissues had a longer T1 relaxation time than the surrounding tissues. Significant decrease of T1 values was observed at the periphery of the DU145 tumor in the T1 map after the injection of the targeted conjugate, while no significant changes of the T1 values was found in DU-145 tumor with the non-targeted agent and the SLK tumor for both conjugates. The RGD targeted conjugate had higher binding affinity to the DU145 cells than the SLK cells, resulting in more effective accumulation and significant T1 shortening at the periphery of the DU145 tumor. The binding of the targeted conjugate was trivial in the SLK tumor and little accumulation of the non-targeted conjugate was found in both tumors. No significant decrease of T1 values was observed in the core of DU145 tumor with the targeted agent because of necrosis and inefficient delivery of the agent to the inner tumor tissue. No significant enhancement was observed in the T1-weighted image of The DU-145 tumor with the targeted conjugate, possibly due to the T1* effect and T2 shortening by the agent.

Conclusions. The results have demonstrated that the RGD targeted PGA-cystamine-(Gd-DOTA) conjugate is effective to bind to the angiogenesis biomarker αvβ3 integrin. Quantitative T1 mapping is a more sensitive tool to detect the biomarker with the targeted agent than conventional contrast enhanced MRI.