I/^{125}I-RGD-DOTA-Gd as a Potential $\alpha_v\beta_3$ -integrin Targeting Dual MRI/SPECT Agent

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Introduction

The combination of magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) for cancer diagnosis have several attractive attributes, as well as overcome the limited sensitivity of MRI. Imaging contrast agent with single form can help to ensure the same pharmacokinetics and colocalization of signal for each modality. Compared to nano-platform, a small molecule based probe has the advantage of applying clinical trial. RGD peptide is well known to have a relatively high and specific affinity for $\alpha_v\beta_3$ integrin, over-expressed in nascent endothelial cells during angiogenesis in various tumor types. In this paper, we introduce the first I/^{125}I-YKRGD-DOTA-Gd complexes (Fig. 1) as a potential αvβ3-integrin targeting dual MRI/SPECT contrast agent that is designed to monitor the activation of tumor. We report here conditions for the preparation and evaluation of this agent in an U87MG glioblastoma xenograft model.

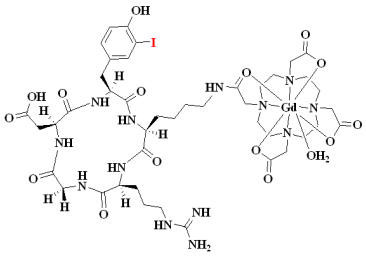

Figure 1 *Structure of I-YKRGD-DOTA-Gd and ^{125}I-YKRGD-DOTA-Gd (I=^{125}I).*

Material and Methods

I-YKRGD was purchased from Anygene. I-YKRGD-DOTA-Gd was synthesized as previously described. YKRGD-DOTA-Gd was labeled with^{125}I using the chloramin-T method. [^{125}I]NaI (1 mCi) was added to the YKRGD-DOTA-Gd (50 μg/50 μL) and mixed at RT. After 2 min, the iodination was quenched with $Na_2S_2O_5$ (500 μg/50 μL). The reaction mixture was analyzed by HPLC using a C-18 semipreparative column with a 0.1% TFA in acetonitrile/0.1% TFA in water from 5/95 (v/v) to 40/60(v/v) at a flow rate of 1 mL/min. The in vitro serum stability of these complexes was conducted using HPLC. For the in vivo study, the U87MG tumor model was generated by subcutaneous injections of 5×10^6 cells in the right flank of ICR mouse (30 g). MR images of mice were obtained pre- and post- I-YKRGD-DOTA-Gd (0.1 mmol Gd/kg) injection by tail vein with a 3 T MR (Magnetom Trio Tim, Siemens). The imaging parameters for T1WI were as follows: TR = 9.70 ms; TE = 3.61 ms; 60 mm FOV; 256 × 256 matrix size; 1 mm slice thickness; NEX = 4. Multi pin-hole SPECT images (70 min) was obtained after injecting (^{125}I-YKRGD-DOTA-Gd, 300 μCi), using multi-modal SPECT/CT system (INVEON, Siemens Medical Solutions). To evaluate the distribution of these complexes, ^{125}I-YKRGD-DOTA-Gd (10 μCi) administered into the tail vein of the mice. The percent injected dose per gram (%ID/g) was determined.

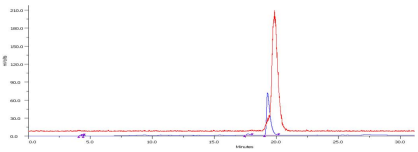

Figure 2 *HPLC of I-YKRGD-DOTA-Gd (blue) and ^{125}I-YKRGD-DOTA-Gd (red).*

Results and Discussion

The formation of I/^{125}I-YKRGD-DOTA-Gd was confirmed by analytical and spectroscopic techniques. The I-YKRGD-DOTA-Gd was characterized by mass spectrum, MALDI-TOF-MS: m/z = 1286.2 ($C_{43}H_{63}GdIN_{13}O_{15}$, Calculated MW = 1286.29). ^{125}I-YKRGD-DOTA-Gd was obtained with high radio chemical purity and characterized by comparing its HPLC profile with that of the corresponding I-YKRGD-DOTA-Gd. Co-injection of a mixture of I /^{125}I-YKRGD-DOTA-Gd would give peaks at the same retention time (Fig. 2), demonstrating their chemical and structural equivalence. The stability assay on I/^{125}I-YKRGD-DOTA-Gd was conducted by monitoring the signals from a UV absorbance detector at 270 nm and γ-trace respectively on the HPLC. I/^{125}I-YKRGD-DOTA-Gd remains stable enough in mouse serum for as long as 3 days. The in vivo MR images of mice obtained with I-YKRGD-DOTA-Gd shows a significant enhancement in the tumor. To further establish the specificity of our tumor-targeting Gd-DOTA-RGD, we performed additional receptor blocking experiments as follows: The Mice were initially injected with c(RGDYK) (10 mg) for blocking the $\alpha_v\beta_3$ receptor and subsequently with I-YKRGD-DOTA-Gd after 30 min, and images taken under the same experimental condition as described above. No significant contrast enhancement in the MR image was observed in the tumor after injection (Fig. 3), demonstrating that I-YKRGD-DOTA-Gd is capable of targeting specifically the $\alpha_v\beta_3$ receptor. The in vivo SPECT images with ^{125}I-YKRGD-DOTA-Gd showed high uptake of tumor (Fig. 4), that was general agreement with the results of the in vivo MR images. The enhancement of kidney indicates that elimination of these complexes take place mainly through glomerular filtration. The results of biodistribution study show that the highest levels of ^{125}I-YKRGD-DOTA-Gd uptake were found in the kidneys and then tumors.

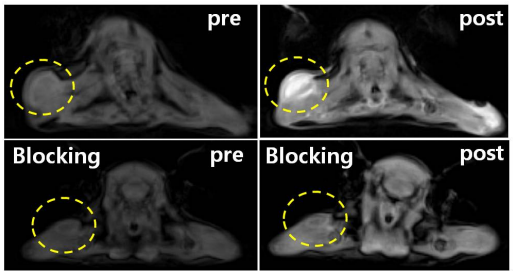

Figure 3 *In vivo MR images of I-YKRGD-DOTA-Gd (up), and RGD-blocking (down) in mice bearing U87MG tumors.*

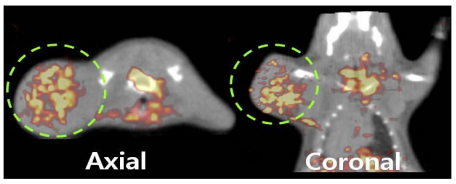

Figure 4 *In vivo SPECT of ^{125}I-YKRGD-DOTA-Gd*

Conclusion

This work describes the successful synthesis and application of I/^{125}I-YKRGD-DOTA-Gd for the $\alpha_v\beta_3$ receptor. The in vivo MR and SPECT images of mice obtained with these complexes are coherent, showing strong enhancement in tumor. This is the demonstration of possible application of I/^{125}I-YKRGD-DOTA-Gd as a tumor targeting contrast agent for MR/SPECT dual imaging.