Aminocyclopentane- and Aminocyclophexane-carboxylic acid containing cyclic RGD-DOTA-Gd Conjugates with high specific affinity for $\alpha_v\beta3$ integrin as MRI contrast agents

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Introduction

RGD peptide has been well known to have a relatively high and specific affinity for $\alpha_v\beta_3$ integrin, which is over-expressed during angiogenesis in various tumor types. Gadolinium (Gd) complexes conjugated with RGD are attractive candidates as paramagnetic contrast agents (CAs) for tumor-targeting in the *in vivo* magnetic resonance imaging (MRI).[1] In this study, we designed two integrin $\alpha_v\beta_3$-specific MRI contrast agents (Figure 1), in which aminocyclopentane (ACP) or amicocyclohexane (ACH) -carboxylic acid is inserted into c(RGDK) to improve the binding affinity and evaluated their tumor targeting efficacy in U87MG tumor bearing mice.

Material and Methods

The c(RGD) peptide conjugates, c(RGD-ACP-K)-DOTA and c(RGD-ACH-K)-DOTA, were custom synthesized by Anygen, Inc. (Korea). The DOTA-Gd complex was prepared as described previously. [1] The receptor-binding assay of the c(RGD-ACP-K)-DOTA-Gd (**1**) and c(RGD-ACH-K)-DOTA-Gd (**2**) were compared with c(RGDyK) using ^{125}I-echistatin on U87MG cells. For the *in vivo* study, the U87MG tumor model was generated by subcutaneous injections of 5×10^6 cells in the right flank of nude mouse (25 g). MR images of mice were obtained pre- and post 1 or 2 (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.

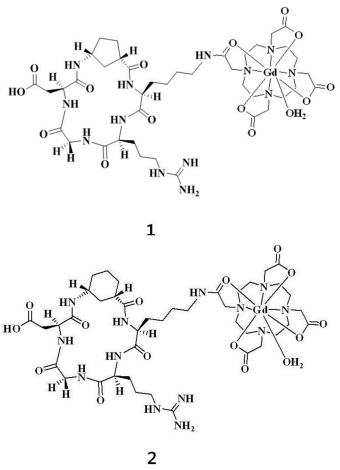

Figure 1 Structure of **1** and **2**.

Results and Discussion

The formation of **1** and **2** were confirmed by MALDI-mass spectroscopy and the purity of the conjugates were obtained in high yields (>95%) after HPLC purification. The mass spectroscopy data for **1** and **2** were m/z = 1110.5 ($C_{40}H_{67}GdN_{13}O_{14}$, Calculated MW = 1111.3) and 1122.3 ($C_{41}H_{66}GdN_{13}O_{14}$, Calculated MW = 1122.2), respectively. On the basis of the cell binding assay, the IC50 values followed the order of c(RGD-ACP-K)-DOTA>c(RGD-ACH-K)-DOTA>c(RGDyK) (Figure 2). The proton relaxivities- r_1 of **1**, **2** and Dotarem® are 5.1±0.1, 5.5±0.1 and 3.7±0.1 $mM^{-1}s^{-1}$, respectively at 128 MHz. It is worth nothing that the r_1 value of **1** and **2** are twice as high as that of Dotarem®. Slower tumbling motion in both complexes as a result of an increase in molecular weight achieved through conjugation with RGD may partially explain such increases in relaxivities as compared with Dotarem®. The *in vivo* MR images of mice obtained with **1** and **2** shows a significant enhancement in the tumor (Figure. 3). To further establish the molecular specific tumor imaging of **1** and **2**, 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 **1** or **2** 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, demonstrating that both complexes are capable of targeting specifically the $\alpha_v\beta_3$ receptor.

Conclusion

We could synthesize modified c(RGD) conjugates for the $\alpha_v\beta_3$ receptor for *in vivo* MR images, and obtain coherent in vivo mice MR images showing strong enhancement in tumor. These results reveal that potential of 1 and 2 as a tumor targeting contrast agent for MR imaging.

[1] Park, J. A.; Lee, J. J.; Jung, J. C; Yu, D. Y.; Oh, C.; Ha, S.; Kim, T. J.; Chang, Y. *ChemBioChem.* **2008**, *9*, 2811-2813.

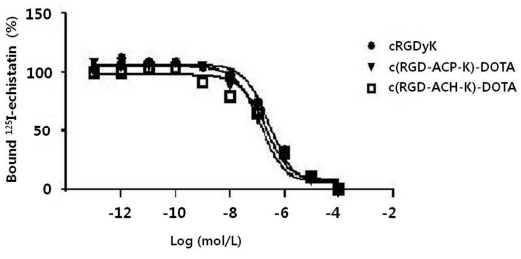

Figure 2 The affinities of c(RGDyK), c(RGD-ACP-K)-DOTA and c(RGD-ACH-K)-DOTA.

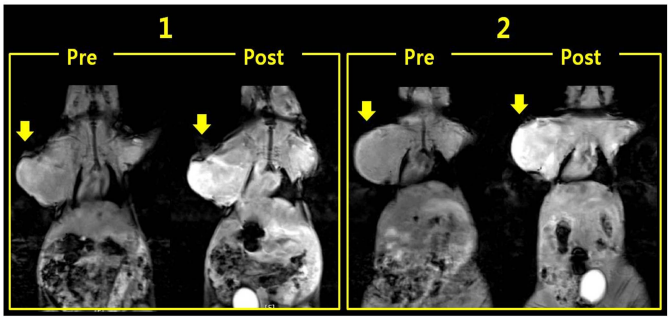

Figure 3 *In vivo* MR images of **1** and **2** in mice bearing U87MG tumors.