DOI: 10.1002/cbic.200800529

Gd-DOTA Conjugate of RGD as a Potential Tumor-Targeting MRI Contrast Agent

Ji-Ae Park,[a] Jae-Jun Lee,[a] Jae-Chang Jung,[b] Dae-Yeul Yu,[c] Chilhwan Oh,[d, e] Seunghan Ha,[e] Tae-Jeong Kim,*[f] and Yongmin Chang*[a, g]

Targeted delivery of contrast agents (CAs) with specific tumor recognition sites and simultaneous monitoring of the growth and metastasis of tumors in the body is an important goal in diagnostic molecular imaging. RGD (Arg-Gly-Asp) peptide is well known to have a relatively high and specific affinity for $\alpha_v\beta_3$-integrin, which is over-expressed in nascent endothelial cells during angiogenesis (formation of new blood vessels) in various tumor types and not in inactive endothelial cells. The expression of an endothelial $\alpha_v\beta_3$-integrin has been shown to correlate with tumor grade and thus plays a significant role in diagnosis of tumors.[1]

Some progress in tumor-targeted imaging by positron emission tomography (PET)[2] or near-infrared fluorescence (NIRF) has recently been made with the aid of RGD complexes labeled with radioactive isotopes or fluorescent tags.[3] Despite the usefulness of PET and NIRF, their applications are rather limited because of inherent problems such as light scattering, the invasive nature of data collection, photo-bleaching, and poor resolution. Molecular magnetic resonance imaging (MRI), however, can not only overcome these restrictions, but, with the assistance of a CA that catalytically shortens the relaxation time of the protons of nearby water molecules, can also provide excellent anatomy images.[4]

[a] J.-A. Park, J.-J. Lee, Prof. Y. Chang
Department of Medical and Biological Engineering
Kyungpook National University
Daegu 702-701 (Korea)

[b] Prof. J.-C. Jung
Department of Biology, Kyungpook National University
Daegu 702-701 (Korea)

[c] Dr. D.-Y. Yu
Korea Research Institute of Bioscience and Biotechnology
Daejeon 305-806 (Korea)

[d] Prof. C. Oh
Department of Dermatology, College of Medicine, Korea University
Seoul 152-703 (Korea)

[e] Prof. C. Oh, S. Ha
Research Institute for Skin Image College of Medicine, Korea University
Seoul 152-703 (Korea)

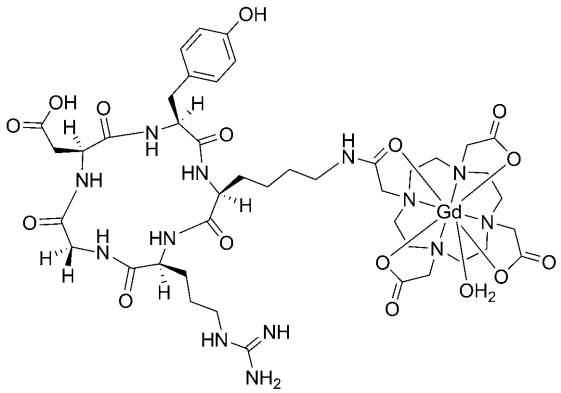
[f] Prof. T.-J. Kim
Department of Applied Chemistry, Kyungpook National University
Daegu 702-701 (Korea)
Fax: (+82) 53-950-6594
E-mail: tjkim@knu.ac.kr

[g] Prof. Y. Chang
Department of Diagnostic Radiology and Molecular Medicine
Kyungpook National University
Daegu 702-701 (Korea)
Fax: (+82) 53-422-2677
E-mail: ychang@knu.ac.kr

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

Thermodynamically stable, water-soluble, and highly paramagnetic Gd^{III} complexes, each bearing a multidentate ligand and at least one coordinated water molecule, have demonstrated high relaxivity and have therefore served as versatile MRI CAs.[5] Among the early MRI CAs approved for clinical use are Dotarem® and Omniscan®. These Gd^{III} complexes incorporate the macrocycle 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as ligand and exhibit high thermodynamic stability.[6] The fact that DOTA can employ at least one carboxylate to form a conjugate with a peptide such as RGD provides additional advantages for the preparation of target-specific MRI CAs. In this regard, Gd(DOTA) conjugated with RGD should be an attractive candidate as a paramagnetic MRI CA for tumor-targeting.

We now therefore wish to introduce the Gd(DOTA) conjugate of RGD, designed to monitor the activation of $\alpha_v\beta_3$-integrin in tumor tissue. The synthesis initially involved conjugation



Gd-DOTA-RGD

of DOTA and the cyclic pentapeptide c(RGDYK) as described by others.[7] The DOTA-RGD conjugate thus prepared was purified and isolated by preparative HPLC. The Gd-DOTA-RGD complex was prepared by treatment of the DOTA-RGD with $GdCl_3 \cdot 6H_2O$ in water. The final product was isolated as a white solid after purification by preparative HPLC. MALDI-TOF-MS shows a peak corresponding to $[M+H-H_2O]^+$ (*m/z* 1161.50; calculated M_W for $C_{43}H_{67}GdN_{13}O_{16} = 1178.39$).

The proton relaxivities—R_1 and R_2—of Gd-DOTA-RGD are $7.4 \pm 0.20\ mM^{-1}s^{-1}$ and $4.0 \pm 0.24\ mM^{-1}s^{-1}$, respectively at 298 K and 64 MHz. Gd-DOTA-RGD exhibits higher longitudinal relaxivity than small-molecule MRI CAs (for the data see the Supporting Information), which may be explained in terms of slower molecular tumbling (τ_γ) as a result of the increase in molecular weight achieved through conjugation with RGD. In

general, it is well documented in the literature that efficient enhancement in water proton relaxivities can be achieved in macromolecular MRI CAs such as dendrimer-based CAs, biocompatible polymer tagged CAs, etc.[8]

The in vivo tumor targeting ability of Gd-DOTA-RGD for $\alpha_v\beta_3$-integrin was examined with the hepatocellular carcinoma in H-*ras*12V transgenic mice.[9] For MR imaging in the targeting experiments of tumor-bearing mice ($N=3$), the animal model under anesthesia was injected with Gd-DOTA-RGD at a dosage of 1.43 mmol kg^{-1} into the tail vein, and T_1-weighted images were acquired over 270 min. Comparison of the pre- and post-injection MR images in Figures 1 A and B, respectively, shows a

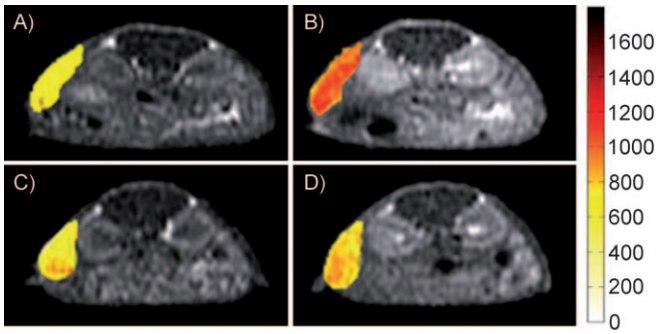


Figure 1. A), C) pre- and B), D) postinjection MR images of mice with hepatocellular carcinoma obtained from the targeting (A, B) and blocking (C, D) experiments. The color indicates the signal intensity according to the pseudo-color scale on the right.

significant enhancement of the MR signals in the tumor and kidney after injection. To establish the specificity of our tumor-targeting Gd-DOTA-RGD, we performed receptor-blocking experiments ($N=3$). The mice were initially injected with c-(RGDYK) (1.43 mmol kg^{-1}) to block the $\alpha_v\beta_3$ receptor and subsequently, after 30 min, with Gd-DOTA-RGD (1.43 mmol kg^{-1}), and images were taken under the same experimental conditions as described above. The differences in the signal intensities as presented in Figures 1 B–D and 2, along with the post-mortem ICP analysis of the tumor (Supporting Information),

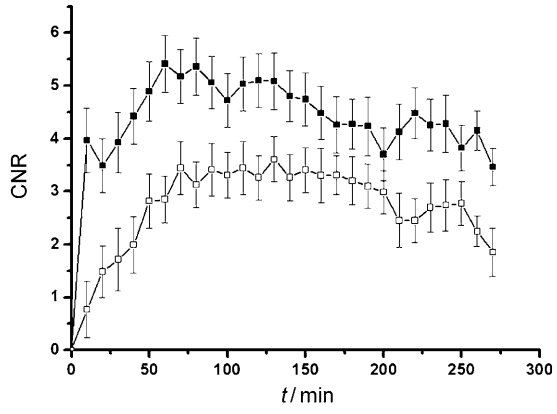


Figure 2. Contrast-to-noise ratio (CNR) as a function of time measured from the targeting (■) and blocking (□) experiments.

clearly demonstrate that Gd-DOTA-RGD is capable of targeting the $\alpha_v\beta_3$ receptor in the tumor cells specifically.

The target-specific nature of Gd-DOTA-RGD may be further confirmed by comparing the normalized signal intensity of Gd-DOTA-RGD as a function of time with that of Omniscan. Figure 3 demonstrates that the normalized signal for Gd-DOTA-RGD remains almost steady throughout 270 min, while

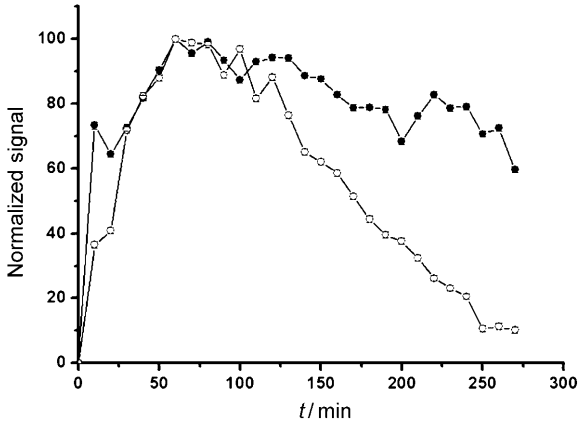


Figure 3. Normalized signal intensities of the tumor as a function of time measured from the in vivo imaging experiments with Gd-DOTA-RGD (●) and Omniscan (○) as the MRI CAs.

that for Omniscan starts decreasing from about 100 min. It may be of interest to mention here that the concentration of the $\alpha_v\beta_3$ receptor in the cells is noticeably low. The integrin receptor is a heterodimeric molecule, comprised of α_v and β_3 subunits, and the concentrations of the α_v and β_3 subunits are 3×10^3–1.4×10^4 per cell and 5.3×10^2–1.1×10^4 per cell, respectively.[10] Thus, in order to induce an observable contrast in the MR image a relatively large concentration of Gd has to be loaded per receptor, and the concentration of Gd used by us seems to be sufficient to achieve that purpose.[11]

The contrast enhancement at the kidney indicates that elimination of Gd-DOTA-RGD takes place mainly through glomerular filtration, as confirmed by Figure 1 B and D. Figure 2 illustrates the CNR of the tumor as a function of time. The CNR increases during the initial 70 min after injection of Gd-DOTA-RGD in the targeting experiment. The blocking experiments also exhibit a similar pattern in the CNR, but approximately 45% lower than that obtained from the targeted experiments. The histological analysis shows that the tumors of all the mice exhibit almost the same vascular density (Supporting Information). All in all, our Gd-DOTA-RGD complex proves to be an efficient tumor-targeting MRI CA for the $\alpha_v\beta_3$ receptor.

The cytotoxicity assay was performed with Gd-DOTA-RGD as well as with Omniscan on 14-day chick cornea stroma primary cells.[12,13] Figure 4 shows no obvious decrease in cell viability when the cells are exposed for 24 h to various concentrations of Gd-DOTA-RGD ($[M]=0.2$–500 μM). These observations indicate that Gd-DOTA-RGD has very low cytotoxicity and can hence be studied further for clinical usage.

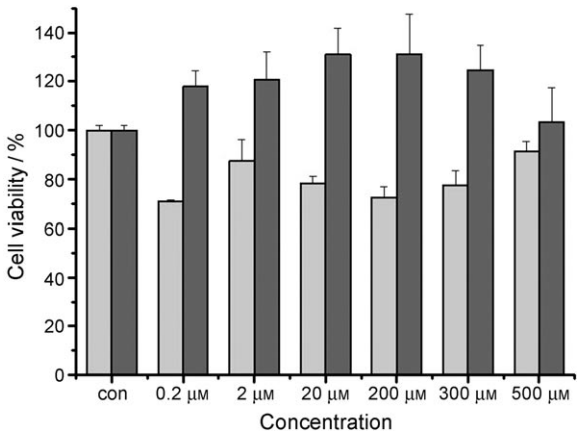


Figure 4. Viabilities of the cells exposed to Gd-DOTA-RGD (dark gray) and Omniscan (gray) at various concentrations.

In summary, this work describes the synthesis and the successful application of Gd-DOTA-RGD as a potential tumor-targeting, nontoxic MRI CA for the $\alpha_v\beta_3$ receptor. This complex exhibits not only higher R_1 relaxivity but moderately good specificity for the $\alpha_v\beta_3$ receptor in hepatocellular carcinoma in H-*ras*12V transgenic mice.

Acknowledgements

The authors gratefully acknowledge the financial support from The Advanced Medical Technology Cluster for Diagnosis and Prediction at KNU from MOCIE, ROK. D.-Y.Y. acknowledges the 21st Century Frontier Program in the Functional Human Genome Project of Korea (HGM0200732). Spectral measurements were performed by the KBSI.

Keywords: Gd chelates · imaging agents · receptors · relaxivity · tumor targeting

[1] a) R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461–7472; b) P. C. Brooks, R. A. F. Clark, D. A. Cheresh, *Science* **1994**, *264*, 569–571; c) W. Arap, R. Pasqualini, E. Ruoslahti, *Science* **1998**, *279*, 377–380; d) A. H. Schmieder, P. M. Winter, S. D. Caruthers, *Magn. Reson. Med.* **2005**, *53*, 621–627; e) R. Pasqualini, E. Koivunen, E. Ruoslahti, *Nat. Biotechnol.* **1997**, *15*, 542–546; f) R. Hwang, J. V. Varner, *Hematol. Oncol.* **2004**, *18*, 991–1006; g) D. A. Sipkins, D. A. Cheresh, M. R. Kazemi, *Nat. Med.* **1998**, *4*, 623–626.

[2] a) Z.-F. Su, G. Liu, S. Gupta, Z. Zhu, M. Rusckowski, D. J. Hnatowich, *J. Bioconjugate Chem.* **2002**, *13*, 561–570; b) R. Haubner, B. Kuhnast, C. Mang, W. A. Weber, H. Kessler, H. J. Wester, M. Schwaiger, *Bioconjugate Chem.* **2004**, *15*, 61–69; c) I. Dijkgraaf, J. A. W. Kruijtzer, C. Frielink, A. C. Soede, H. W. Hilbers, W. J. G. Oyen, F. H. M. Corstens, R. M. J. Liskamp, O. C. Boerman, *Nucl. Med. Biol.* **2006**, *33*, 953–961.

[3] a) Z. Cheng, Y. Wu, Z. Xiong, S. S. Gambhir, X. Chen, *Bioconjugate Chem.* **2005**, *16*, 1433–1441; b) X. Chen, P. S. Conti, R. A. Moats, *Cancer Res.* **2004**, *64*, 8009–8014; c) Y. Wu, W. Cai, X. Chen, *Mol Imaging Biol.* **2006**, *8*, 226–236.

[4] a) A. E. Merbach, E. Tóth, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Wiley, Chichester, **2001**; b) M. Rudin, N. Beckmann, R. Porszasz, T. Reese, D. Bochelen, A. Sauter, *NMR Biomed.* **1999**, *12*, 69–97.

[5] a) P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293–2352; b) S. Aime, M. Botta, M. Fasano, E. Terrono, *Chem. Soc. Rev.* **1998**, *27*, 19–29.

[6] a) J. C. Bousquet, S. Saini, D. D. Stark, P. F. Hahn, M. Nigam, J. Wittenberg, J. T. Ferrucci, Jr., *Radiology* **1988**, *166*, 693–698; b) M. Li, C. F. Meares, *Bioconjugate Chem.* **1993**, *4*, 275–283; c) M. R. Lewis, A. Raubitschek, J. E. Shively, *Bioconjugate Chem.* **1994**, *5*, 565–576.

[7] X. Chen, R. Park, M. Tohme, A. H. Shahinian, J. R. Bading, P. S. Conti, *Bioconjugate Chem.* **2004**, *15*, 41–49.

[8] a) P. Caravan, *Chem. Soc. Rev.* **2006**, *35*, 512–523; b) S. J. Ratnakar, V. Alexander, *Eur. J. Inorg. Chem.* **2005**, 3918–3927; c) E. Tóth, L. Helm, A. E. Merbach, *Top. Curr. Chem.* **2002**, *221*, 61–101.

[9] A.-G. Wang, H. B. Moon, M. R. Lee, C. Y. Hwang, K. S. Kwon, S. L. Yu, Y.-S. Kim, M. Kim, J. M. Kim, S. K. Kim, T.-H. Lee, E.-Y. Moon, D.-S. Lee, D.-Y. Yu, *J. Hepatol.* **2005**, *43*, 836–844.

[10] S. Benedetto, R. Pulito, S. G. Crich, G. Tarone, S. Aime, L. Silengo, J. Hamm, *Magn. Reson. Med.* **2006**, *56*, 711–716.

[11] W. J. M. Mulder, G. J. Strijkers, J. W. Habets, E. J. W. Bleeker, D. W. J. van der Schaft, G. Storm, G. A. Koning, A. W. Griffioen, K. Nicolay, *FASEB J.* **2005**, *19*, 2008–2010.

[12] C. Zou, Z. Shen, *J. Pharmacol. Toxicol. Methods* **2007**, *56*, 58–62.

[13] C. Li, Y.-X. Li, G.-L. Law, K. Man, W.-T. Wong, H. Lei, *Bioconjugate Chem.* **2006**, *17*, 571–574.

Received: August 5, 2008
Published online on October 27, 2008

