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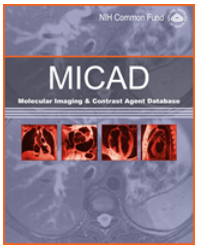

Gadolinium-1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid-icosahedral *closo*-borane$_{12}$ scaffold conjugated with Glu-{Glu-[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$}$_2$

CA-12

Kam Leung, PhD[1]

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Chemical name:	Gadolinium-1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid-icosahedral closo-borane$_{12}$ scaffold conjugated with Glu-{Glu-[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$}$_2$	
Abbreviated name:	CA-12	
Synonym:	Gd-DOTA-*closo*-B$_{12}$-E-{E-[c(RGDfK)]$_2$}$_2$	
Agent category:	Peptide	
Target:	Integrin $\alpha_v\beta_3$	
Target category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Gadolinium, Gd	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Magnetic resonance imaging (MRI) maps information about tissues spatially and functionally (1). Protons (hydrogen nuclei) are widely used in imaging because of their abundance in water molecules. Water comprises ~80% of most soft tissue. The contrast of proton MRI depends primarily on the density of the nucleus (proton spins), the relaxation times of the nuclear magnetization (T1, longitudinal, and T2, transverse), the magnetic environment of the tissues, and the blood flow to the tissues. However, insufficient contrast between normal and

Author Affiliation: 1 National for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: MICAD@ncbi.nlm.nih.gov.

Corresponding author.

diseased tissues requires the development of contrast agents. Most contrast agents affect the T1 and T2 relaxation times of the surrounding nuclei, mainly the protons of water. T2* is the spin–spin relaxation time composed of variations from molecular interactions and intrinsic magnetic heterogeneities of tissues in the magnetic field. Cross-linked iron oxide nanoparticles and other iron oxide formulations affect T2 primarily and lead to decreased signals. On the other hand, paramagnetic T1 agents, such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}), accelerate T1 relaxation and lead to brighter contrast images (2). Gadolinium (Gd), a lanthanide metal ion with seven unpaired electrons, has been shown to be very effective in enhancing proton relaxation because of its high magnetic moment and water coordination (3, 4). Gd-Labeled diethylenetriamine pentaacetic acid (Gd-DTPA) was the first intravenous MRI contrast agent used clinically, and a number of similar Gd chelates have been developed in an effort to further improve clinical use.

Integrins are a family of heterodimeric glycoproteins on cell surfaces that mediate diverse biological events involving cell–cell and cell–matrix interactions (5). Integrins consist of an α and a β subunit and are important for cell adhesion and signal transduction. The $\alpha_v\beta_3$ integrin is the most prominent receptor affecting tumor growth, tumor invasiveness, metastasis, tumor-induced angiogenesis, inflammation, osteoporosis, and rheumatoid arthritis (6-10). Expression of the $\alpha_v\beta_3$ integrin is strong on tumor cells and activated endothelial cells, whereas expression is weak on resting endothelial cells and most normal tissues. Antagonists of $\alpha_v\beta_3$ are being studied as antitumor and antiangiogenic agents, and the agonists of $\alpha_v\beta_3$ are being studied as angiogenic agents for coronary angiogenesis (8-12). Extracellular matrix proteins (vitronectin, fibrinogen, laminin, and collagen) contain a tripeptide sequence consisting of Arg-Gly-Asp (RGD), which binds to a variety of integrins, including $\alpha_v\beta_3$. Various radiolabeled antagonists have been introduced for the imaging of tumors and tumor angiogenesis (13).

Most cyclic RGD peptides are composed of five amino acids. Haubner et al. (14) reported that various cyclic RGD peptides exhibit selective inhibition of binding to $\alpha_v\beta_3$ (inhibition concentration (IC_{50}), 7–40 nM) but not to integrins $\alpha_v\beta_5$ (IC_{50}, 600–4,000 nM) or $\alpha_{IIb}\beta_3$ (IC_{50}, 700–5,000 nM). Various radiolabeled cyclic RGD peptides have been found to have high accumulation in xenografts in nude mice (15, 16). Several small molecular MRI Gd-based contrast agents conjugated with a cyclic RGD peptide have been studied (17). However, these low molecular weight Gd chelates have short blood and tissue retention times, which limit their use as imaging agents in the vasculature and cancer. Furthermore, these low molecular weight MRI contrast agents exhibit low r_1 relaxivity value (~4 $mM^{-1}s^{-1}$ per Gd) and high toxicity (nephrogenic systemic fibrosis in patients with renal dysfunction) (18). In order to obtain sufficient contrast enhancement, a large number of Gd^{3+} ions bound per target is needed. Li et al. (19) have developed polyhedral boranes as a scaffold for the targeted high payload delivery of drugs and imaging agents. A functionalizable monodisperse molecular borane scaffold, $[closoB_{12}(OH)_{12}]^{2-}$, conjugated with twelve radial ethylene glycol $(PEG)_3$ arms with attachments of Gd-chelates (20). Goswami et al. (21) utilized $[closoB_{12}(OH)_{12}]^{2-}$ as a high payload MRI contrast agent (CA-12) carrying eleven copies of Gd-DOTA chelates and one copy of Glu-Glu-{[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$}$_2$ (cRGD) *via* PEG linkers for use as a high-performance MRI contrast agent in nude mice bearing human breast cancer xenografts.

Related Resource Links:

- Chapters in MICAD (Gd, RGD)
- Gene information in NCBI (α_v integrin, β_3 integrin)
- Articles in Online Mendelian Inheritance in Man (OMIM) (α_v integrin, β_3 integrin)
- Clinical trials (Gd-DTPA, Gd-DOTA)
- Drug information in FDA (Gd-DTPA, Gd-DOTA)

Synthesis

[PubMed]

The 12-fold carbamate closomer 5 was reacted with amino-terminated DOTA-$(PEG)_5$ ligand 4 to form 12-fold DOTA-conjugated closomer 6 with 80% yield (21). Closomer 6 was isolated with column chromatography and then treated with trifluoroacetic acid (TFA) to remove the *tert*-butyl ester protecting groups. The deprotected closomer 6 was incubated with $GdCl_3$ in a citrate buffer (pH 7) to provide closomer CA-7 with 71% yield. CA-7 was purified with dialysis in ultrapure water. There were 11.4 Gd^{3+} ions per CA-7 as measured with inductively coupled plasma optical emission spectroscopy (ICP-OES). The average CA-7 particle diameter was 10 nm as measured with dynamic laser scattering (DLS).

The 11-fold carbonate closomer 9 with an azido-terminated $(PEG)_8$ linker was reacted with alkyne-terminated $(PEG)_9$ cRGD peptide by an azide-alkyne click reaction (21). The resulting cRGD-closomer 10 was isolated with column chromatography. DOTA ligands were added to the remaining 11 vertices by reaction with amino-terminated DOTA-$(PEG)_5$ ligand 4 to obtain the 11-fold carbamate closomer 11 in 83% yield. Closomer 11 was isolated with column chromatography and then treated with TFA to remove the *tert*-butyl ester protecting groups. The deprotected closomer 11 was incubated with $GdCl_3$ in a citrate buffer (pH 7) to provide closomer CA-12 with 69% yield. CA-12 was purified with dialysis in ultrapure water. There were 10.6 Gd^{3+} ions per CA-12 as measured with ICP-OES. The average CA-12 particle diameter was 12–13 nm as measured with DLS.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

CA-7 exhibited an r_1 value (7 T) of 4.8 $mM^{-1}s^{-1}$ per Gd in phosphate-buffered saline (PBS) to provide a molar relaxivity value of 54.7 $mM^{-1}s^{-1}$ (21). CA-12 exhibited an r_1 value of 5.9 $mM^{-1}s^{-1}$ per Gd to provide a molar relaxivity value of 62.5 $mM^{-1}s^{-1}$. Omniscan (Gd-DTPA-BMA) had a molar relaxivity value of 4.2 $mM^{-1}s^{-1}$. Cellular accumulation of CA-12 was performed by incubation of CA-9 (5 mM Gd) with MDA-MB-231 ($\alpha_v\beta_3$-positive) and T47D ($\alpha_v\beta_3$-negative) cells for 30 min at 37°C. The Gd concentration was 200% higher in MDA-MB-231 cells (90 μg Gd/g cell lysate) than in T47D cells (30 μg Gd/g cell lysate). No blocking studies were performed.

Animal Studies

Rodents

[PubMed]

Goswami et al. (21) performed dynamic T1-weighted MRI (7 T) studies with intravenous injection of 0.04 mmol Gd/kg CA-12, CA-7, or Gd-DTPA-BMA in nude mice (n = 6/group) bearing PC-3 xenografts. Contrast-enhanced MRI signal intensity (SI) levels were obtained at 1 min, 30 min, 1 h, 4 h, and 24 h after intravenous injection. Enhanced contrast in the tumor tissues was visualized for CA-12 for up to 4 h, whereas contrast enhancement with CA-7 persisted for only 1 h after injection. On the other hand, Gd-DTPA-BMA exhibited contrast enhancement within 30 min, and little enhancement was detected after 1 h. Contrast enhancement ratios (CERs) [CER = $(SI_{post} - SI_{pre})/SI_{pre}$ X 100%] for CA-12 were 16%, 27%, 24%, 17%, and 3% at 1 min, 30 min, 1 h, 4 h, and 24 h, respectively. CERs for CA-7 were 13%, 18%, 16%, 10%, and 3% at 1 min, 30 min, 1 h, 4 h, and 24 h, respectively. The CERs of tumors for Gd-DTPA-BMA were 32%, 18%, 4%, 4%, and 3% at 1 min, 30 min, 1 h, 4 h, and 24 h, respectively. At 1 h and 4 h after injection of CA-12, tumor tissues exhibited significant higher CERs compared to those with CA-7 ($P < 0.01$ at 1 h) and Gd-DTPA-BMA ($P < 0.01$ at 1 h, $P < 0.05$ at 4 h). The three agents showed high CERs of 90%–120% in the kidneys at 1 min, which declined to ~3% at 24 h.

CERs for CA-12 and CA-7 declined more slowly than that for Gd-DTPA-BMA. The Gd contents in the kidneys as well as other major normal tissues were not detectable for the three agents by 24 h after injection. No blocking studies were performed.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

R21 CA114090

References

1. Wang Y.X., Hussain S.M., Krestin G.P. *Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging.* . Eur Radiol. 2001;11(11):2319–31. PubMed PMID: 11702180.
2. Burtea, C., S. Laurent, L. Vander Elst, and R.N. Muller, *Contrast agents: magnetic resonance.* Handb Exp Pharmacol, 2008(185 Pt 1): p. 135-65.
3. Brasch R.C. *New directions in the development of MR imaging contrast media.* . Radiology. 1992;183(1):1–11. PubMed PMID: 1549653.
4. Runge V.M., Gelblum D.Y. *Future directions in magnetic resonance contrast media.* . Top Magn Reson Imaging. 1991;3(2):85–97. PubMed PMID: 2025435.
5. Hynes R.O., Lively J.C., McCarty J.H., Taverna D., Francis S.E., Hodivala-Dilke K., Xiao Q. *The diverse roles of integrins and their ligands in angiogenesis.* . Cold Spring Harb Symp Quant Biol. 2002;67:143–53. PubMed PMID: 12858535.
6. Grzesik W.J. *Integrins and bone--cell adhesion and beyond.* . Arch Immunol Ther Exp (Warsz). 1997;45(4):271–5. PubMed PMID: 9523000.
7. Jin H., Varner J. *Integrins: roles in cancer development and as treatment targets.* . Br J Cancer. 2004;90(3):561–5. PubMed PMID: 14760364.
8. Kumar C.C. *Integrin alpha v beta 3 as a therapeutic target for blocking tumor-induced angiogenesis.* . Curr Drug Targets. 2003;4(2):123–31. PubMed PMID: 12558065.
9. Ruegg C., Dormond O., Foletti A. *Suppression of tumor angiogenesis through the inhibition of integrin function and signaling in endothelial cells: which side to target?* . Endothelium. 2002;9(3):151–60. PubMed PMID: 12380640.
10. Wilder R.L. *Integrin alpha V beta 3 as a target for treatment of rheumatoid arthritis and related rheumatic diseases.* . Ann Rheum Dis. 2002;61 Suppl 2:ii96–9. PubMed PMID: 12379637.
11. Kerr J.S., Mousa S.A., Slee A.M. *Alpha(v)beta(3) integrin in angiogenesis and restenosis.* . Drug News Perspect. 2001;14(3):143–50. PubMed PMID: 12819820.

12. Mousa S.A. *alphav Vitronectin receptors in vascular-mediated disorders.* . Med Res Rev. 2003;23(2):190–9. PubMed PMID: 12500288.
13. Haubner R., Decristoforo C. *Radiolabelled RGD peptides and peptidomimetics for tumour targeting.* . Front Biosci. 2009;14:872–86. PubMed PMID: 19273105.
14. Haubner R., Wester H.J., Burkhart F., Senekowitsch-Schmidtke R., Weber W., Goodman S.L., Kessler H., Schwaiger M. *Glycosylated RGD-containing peptides: tracer for tumor targeting and angiogenesis imaging with improved biokinetics.* . J Nucl Med. 2001;42(2):326–36. PubMed PMID: 11216533.
15. Beer A.J., Kessler H., Wester H.J., Schwaiger M. *PET Imaging of Integrin alphaVbeta3 Expression.* . Theranostics. 2011;1:48–57. PubMed PMID: 21547152.
16. Zhou Y., Chakraborty S., Liu S. *Radiolabeled Cyclic RGD Peptides as Radiotracers for Imaging Tumors and Thrombosis by SPECT.* . Theranostics. 2011;1:58–82. PubMed PMID: 21547153.
17. Tan M., Lu Z.R. *Integrin Targeted MR Imaging.* . Theranostics. 2011;1:83–101. PubMed PMID: 21547154.
18. Laurent S., Elst L.V., Muller R.N. *Comparative study of the physicochemical properties of six clinical low molecular weight gadolinium contrast agents.* . Contrast Media Mol Imaging. 2006;1(3):128–37. PubMed PMID: 17193689.
19. Li T., Jalisatgi S.S., Bayer M.J., Maderna A., Khan S.I., Hawthorne M.F. *Organic syntheses on an icosahedral borane surface: closomer structures with twelvefold functionality.* . J Am Chem Soc. 2005;127(50):17832–41. PubMed PMID: 16351114.
20. Goswami L.N., Ma L., Chakravarty S., Cai Q., Jalisatgi S.S., Hawthorne M.F. *Discrete nanomolecular polyhedral borane scaffold supporting multiple gadolinium(III) complexes as a high performance MRI contrast agent.* . Inorg Chem. 2013;52(4):1694–700. PubMed PMID: 23126285.
21. Goswami L.N., Ma L., Cai Q., Sarma S.J., Jalisatgi S.S., Hawthorne M.F. *cRGD peptide-conjugated icosahedral closo-B12(2-) core carrying multiple Gd3+-DOTA chelates for alpha(v)beta3 integrin-targeted tumor imaging (MRI).* . Inorg Chem. 2013;52(4):1701–9. PubMed PMID: 23391150.