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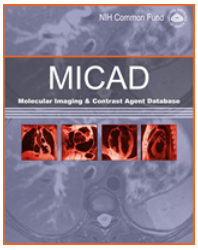


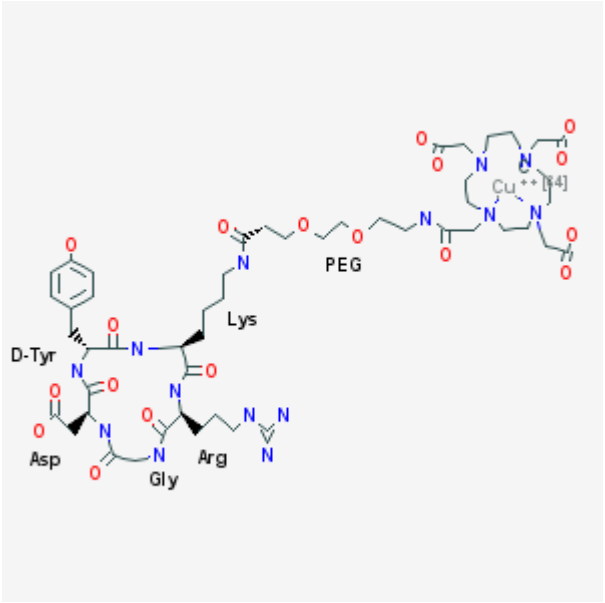
^{64}Cu-1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-PEGylated cyclic arginine-glycine-aspartic acid peptide

^{64}Cu-DOTA-PEG-c(RGDyK)

The MICAD Research Team

Created: May 10, 2007; Updated: July 17, 2007.

Chemical name:	^{64}Cu-1,4,7,10-Tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid-PEGylated cyclic arginine-glycine-aspartic acid peptide
Abbreviated name:	^{64}Cu-DOTA-PEG-c(RGDyK)
Synonym:	^{64}Cu-Pegylated c(RGDyK)
Agent Category:	Peptide
Target:	Integrin $\alpha_v\beta_3$
Target Category:	Receptor binding
Method of detection:	Positron Emission Tomography (PET)
Source of signal:	^{64}Cu
Activation:	No
Studies:	• *In vitro* • Rodents



Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$.

Background

[PubMed]

^{64}Cu-1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-PEGylated cyclic arginine-glycine-aspartic acid peptide [^{64}Cu-DOTA-PEG-c(RGDyK)] is an integrin-targeted molecular imaging agent developed for positron emission tomography (PET) of tumor vasculature and tumor angiogenesis (1). ^{64}Cu is a positron emitter with a physical half-life ($t_{½}$) of 12.8 h.

Cellular survival, invasion, and migration control embryonic development, angiogenesis, tumor metastasis, and other physiological processes (2, 3). Among the molecules that regulate angiogenesis are integrins, which comprise a superfamily of cell adhesion proteins that form heterodimeric receptors for extracellular matrix (ECM) molecules (4, 5). These transmembrane glycoproteins consist of two noncovalently associated subunits, α and β (18 α- and 8 β-subunits in mammals), which are assembled into at least 24 α/β pairs. Several integrins, such as integrin $\alpha_v\beta_3$, have affinity for the arginine-glycine-aspartic acid (RGD) tripeptide motif, which is found in many ECM proteins. Expression of integrin $\alpha_v\beta_3$ receptors on endothelial cells is stimulated by angiogenic factors and environments. The integrin $\alpha_v\beta_3$ receptor is generally not found in normal tissue, but it is strongly expressed in vessels with increased angiogenesis, such as tumor vasculature. It is significantly upregulated in certain types of tumor cells and in almost all tumor vasculature.

Molecular imaging probes that carry the RGD motif that binds to the integrin $\alpha_v\beta_3$ can be used to image tumor vasculature and evaluate angiogenic responses to tumor therapy (6, 7). Various RGD peptides in both linear and cyclic forms (RGDfK or RGDyK) have been developed for *in vivo* binding to integrin $\alpha_v\beta_3$ (8). Chen et al (9). evaluated a cyclic RGD D-Tyr analog peptide [c(RGDyK)] labeled with ^{64}Cu or ^{18}F in nude mice bearing breast tumor. Chen et al. used 1,4,7,10-tetrazadodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) for c(RGDyK) conjugation with ^{64}Cu. ^{64}Cu-DOTA-c(RGDyK) showed prolonged tumor radioactivity retention but persistent liver radioactivity. To improve the pharmacokinetics and tumor retention of the radiolabeled peptide, Chen et al. (10) modified c(RGDyK) with monofunctional methoxy-polyethylene glycol (PEG; molecular weight = 2,000) and showed that the modified PEGylated RGD peptide had faster blood clearance, lower kidney uptake, and prolonged tumor uptake. Using the same strategy, Chen et al. (1) inserted a heterobifunctional PEG linker (molecular weight = 3,400) between DOTA and c(RGDyK) to produce ^{64}Cu-DOTA-PEG-c(RGDyK). The PEG moiety appeared to improve the *in vivo* kinetics of this PET radioligand.

Synthesis

[PubMed]

Chen et al. (1) reported the synthesis of ^{64}Cu-DOTA-PEG-c(RGDyK). The c(RGDyK) peptide was first prepared *via* solution cyclization of fully protected linear pentapeptide H-Gly-Asp(OtBu)-D-Tyr(OtBu)-Lys(Boc)-Arg(Pbf)-OH, followed by trifluoroacetic acid (TFA) deprotection in the presence of the free radical scavenger triisopropylsilane. The c(RGDyK)-PEG conjugate was prepared by coupling c(RGDyK) with t-butoxycarbonyl (t-Boc)-protected PEG-succinimidyl ester (t-Boc-NH-PEG-CO_2Su). The t-Boc protecting group was unblocked by TFA cleavage. DOTA was obtained commercially. The DOTA-PEG-c(RGDyk) conjugate was prepared by *in situ* activation of DOTA by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) and *N*-hydroxysulfonosuccinimide (SNHS) in a molar ratio of 10:5:4 (DOTA:EDC:SNHS).The c(RGDyk)-PEG conjugate in water at 4°C was added to the resultant DOTA-sulfosuccinimydyl ester (OSSu), and the pH was adjusted to 8.5. The reaction mixture was incubated overnight at 4°C. The DOTA-PEG-c(RGDyk) conjugate was then purified by semipreparative high-performance liquid chromatography (HPLC). The molecular weight of the DOTA-PEG-c(RGDyK) conjugate was determined to be 4,400 by mass spectrometry, which appeared to agree with the theoretical value. The purified product was labeled with ^{64}Cu by addition of ^{64}Cu in the ratio of 5–25 μg peptide per 37 MBq (1 mCi) ^{64}Cu in 0.1-N sodium acetate buffer (pH 5.5). This was followed by incubation at 50°C for 45 minutes. The reaction was terminated by addition of ethylenediaminetetraacetic acid (EDTA). ^{64}Cu-DOTA-PEG-c(RGDyK) was purified with a C-18 Sep-Pak cartridge that used 85% ethanol as the elution solvent. The radiochemical purities were 50%, 80%, 95%, and ≥98% for 5, 10, 15, and 20 μg peptide, respectively. The specific activity of ^{64}Cu-DOTA-PEG-c(RGDyK) ranged from 14,800 to 29,600 GBq/mmol (400 to 800 Ci/mmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Solid-phase receptor-binding assays of unlabeled DOTA-PEG-c(RGDyK) were conducted by competitive displacement of ^{125}I-labeled echistatin binding to purified human $\alpha_v\beta_3$ integrin (1). The inhibition concentration (IC_{50}) was 67.5 ± 10.5 nM. In comparison, the IC_{50} for unpegylated DOTA-c(RGDyK) was 31.2 ± 5.8 nM. In a hydrophilicity study, the octanol-water partition coefficient (LogP_{OCT}) for ^{64}Cu-DOTA-PEG-c(RGDyK) was determined to be −2.97 ± 0.30 (n = 3). The LogP_{OCT} for ^{64}Cu-DOTA-c(RGDyK) was −2.8 ± 0.04.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{64}Cu-DOTA-PEG-c(RGDyK) were conducted in nude mice bearing s.c. U87MG glioblastomas (0.4–0.6 cm in diameter) (1). Each mouse received 370 kBq (10 μCi) of ^{64}Cu-DOTA-PEG-c(RGDyK) by i.v. administration. The radioligand was rapidly cleared from the blood and most organs, and the kidneys appeared to be the primary route of excretion. The radioactivity levels (n = 4) in the tumor were 2.74 ± 0.45% injected dose/g (% ID/g) and 1.62 ± 0.18% ID/g at 0.5 h and 4 h, respectively. The blood radioactivity levels were 0.57 ± 0.15% ID/g and 0.02 ± 0.02% ID/g at 0.5 h and 2 h, respectively. The kidney radioactivity level was ~3.2% ID/g (extrapolated from Figure 3) at 0.5 h and decreased to ~1.2% ID/g at 4 h. The liver activity was 0.58 ± 0.07% ID/g at 4 h. Overall, the authors suggested that PEGylation had a minimal effect on tumor uptake and clearance. When compared to the unPEGylated radioligand, the PEGylated radioligand also had a relatively faster renal clearance and a lower liver accumulation. Coinjection of 10 mg/kg unlabeled c(RGDyK) peptide decreased the radioactivity of ^{64}Cu-DOTA-PEG-c(RGDyK) in all tissues except the kidneys. The radioactivity level of the tumor at 1 h was reduced from 2.21 ± 0.38% ID/g to 0.22 ± 0.03% ID/g.

Imaging of 14.8 MBq (400 μCi) ^{64}Cu-DOTA-PEG-c(RGDyK) with microPET at 1 h in nude mice bearing the U87MG tumor clearly visualized the tumor with high contrast relative to the contralateral background (1). The kidneys, intestinal tract, and urinary bladder were also visible on the image. Digital whole-body autoradiography of the mice after PET imaging showed a distribution pattern similar to of the patterns observed in the biodistribution and imaging studies. The radioactivity levels in the tumor, liver, and kidneys were 2.6 ± 0.6% ID/g, 0.8 ± 0.2% ID/g, and 2.4 ± 0.5% ID/g, respectively. In a single mouse, PET imaging also visualized a U87MG tumor implanted into the mouse forebrain with a tumor/brain ratio of 4.0 ± 0.5. However, the absolute tumor radioactivity level was only 0.4 ± 0.1% ID/g.

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.

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