^{64}Cu-Labeled 4-((8-amino-3,6,10,13,16,19-hexaazabicyclo [6.6.6] icosane-1-ylamino)methyl)benzoic acid (AmBaSar) conjugated to cyclic arginine-glycine-aspartic acid (RGD) peptide

[^{64}Cu]-AmBaSar-RGD

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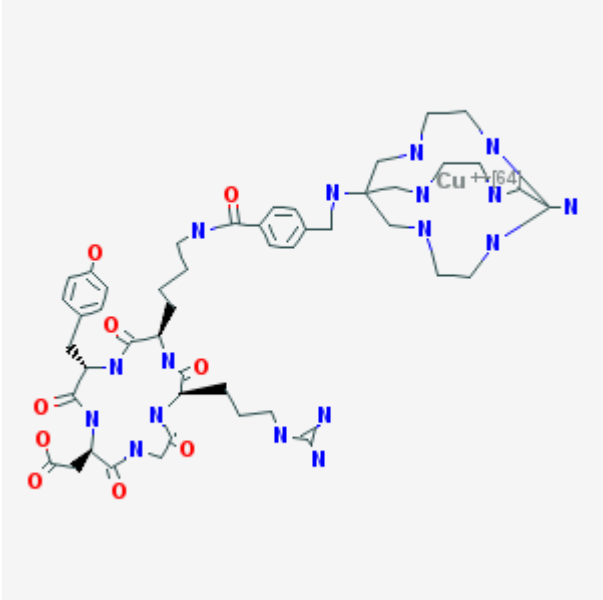
Created: September 20, 2010; Updated: October 28, 2010.

Chemical name:	^{64}Cu-Labeled 4-((8-amino-3,6,10,13,16,19-hexaazabicyclo [6.6.6] icosane-1-ylamino)methyl)benzoic acid (AmBaSar) conjugated to cyclic arginine-glycine-aspartic acid (RGD) peptide
Abbreviated name:	[^{64}Cu]-AmBaSar-RGD
Synonym:	
Agent Category:	Compound
Target:	$\alpha_v\beta_3$ integrin
Target Category:	Receptor
Method of detection:	Positron emission tomography (PET)
Source of signal / contrast:	^{64}Cu
Activation:	No



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Studies:	• *In vitro* • Rodents	Click on the above structure of [^{64}Cu]-AmBaSar-RGD for additional information in PubChem.

Background

[PubMed]

The integrin $\alpha_v\beta_3$ receptor is one of several known biomarkers of cancerous tumor angiogenesis (1) and metastasis (2). Therefore, molecular imaging (MI) of integrin $\alpha_v\beta_3$ receptor expression in tumors is considered to be useful for the detection of malignant tumors and the development of an individualized treatment and monitoring regimen for the patient (3). Ligands such as vitronectin, fibronectin, etc., which interact with integrins, are known to bind these receptors through an Arg-Gly-Asp (RGD) epitope. Consequently, many MI probes used for the detection and quantification of integrins with positron emission tomography (PET) or multimodal imaging are based on an RGD motif containing a peptide or its derivative and are labeled with ^{18}F or are conjugated to a chelator for the binding of ^{64}Cu (3, 4). In this regard, ^{64}Cu can be considered to be a more suitable nuclide than ^{18}F (half-life, ~110 min) for the development of a MI probe that can be used to detect integrin $\alpha_v\beta_3$ receptors because of its longer half-life (12.7 h) because a longer circulation time can be necessary for the penetration of dense tumors (5). Recently a cage-like bifunctional chelator, 4-((8-amino-3,6,10,13,16,19-hexaazabicyclo [6.6.6] icosane-1-ylamino)methyl)benzoic acid (AmBaSar) was developed and conjugated to RGD for labeling with ^{64}Cu ([^{64}Cu]-AmBaSar-RGD) (3). Subsequently, Cai et al. evaluated the biodistribution of [^{64}Cu]-AmBaSar-RGD and its effectiveness in the detection of tumors expressing integrin $\alpha_v\beta_3$ receptors in mice bearing U-87MG cell tumors, which express integrin $\alpha_v\beta_3$ receptors (3).

Other Sources of Information

- Chapters on integrins in MICAD
- Protein and mRNA sequence of human alpha v isoforms 2 integrin
- Articles about integrins in Online Mendelian Inheritance in Man Database (OMIM)

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NLM Citation: Chopra A. ^{64}Cu-Labeled 4-((8-amino-3,6,10,13,16,19-hexaazabicyclo [6.6.6] icosane-1-ylamino)methyl)benzoic acid (AmBaSar) conjugated to cyclic arginine-glycine-aspartic acid (RGD) peptide. 2010 Sep 20 [Updated 2010 Oct 28]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

- Integrin clinical trials in ClinicalTrials.gov
- Integrin related pathways in Pathways Interaction Database
- Natalizumab – an alpha(4)beta(1)-integrin antagonist [PubMed]

Synthesis

[PubMed]

The synthesis of AmBaSar-RGD and its labeling with ^{64}Cu have been described by Cai et al. (3). The radiochemical yield of the labeled compound was >95% with a purity of >99% as determined with high-performance liquid chromatography (HPLC). During radiographic thin-layer chromatography (radio-TLC) analysis, ^{64}Cu remained at the origin and the R_f value of [^{64}Cu]-AmBaSar-RGD was between 0.8 and 1.0 (3). The specific activity of the radiochemical was reported to be 10.1–22.2 GBq/μmol (272.7–599.4 mCi/μmol).

For comparison purposes, ^{64}Cu-labeled 1,4,7,10-tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid (DOTA) conjugated to RGD ([^{64}Cu]-DOTA-RGD) was used in some studies (3). The radiochemical yield, purity, and specific activity of this radiocompound were 80%, 98%, and 10.1–22.2 GBq/μmol (272.7–599.4 mCi/μmol), respectively. The R_f value of [^{64}Cu]-DOTA-RGD on radio-TLC analysis was reported to be between 0.8 and 1.0.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

When [^{64}Cu]-AmBaSar-RGD and [^{64}Cu]-DOTA-RGD were respectively exposed to phosphate-buffered saline (pH 7.4), fetal bovine, or mouse serum, the stability of both tracers was reported to be >95% for up to 24 h as determined with HPLC (3).

The 1-octanol/water partition coefficients of [^{64}Cu]-AmBaSar-RGD and [^{64}Cu]-DOTA-RGD were reported to be −2.44 ± 0.12 and −2.80 ± 0.04, respectively, indicating that the two radiochemicals had a hydrophilic nature (3).

In a ^{125}I-Labeled eschistatin binding assay (125I-eschistatin binds specifically to integrin $\alpha_v\beta_3$ receptors) using U-87MG cells, the cellular binding of the ^{125}I-labeled compound was shown to be inhibited by AmBaSar-RGD and c(RGDyK) (a derivative of RGD) in a dose-dependent fashion (3). The 50% inhibition concentration values of AmBaSar-RGD and c(RGDyK) were determined to be 53.26 ± 0.51 nmol/L and 36.55 ± 0.65 nmol/L, respectively.

In another study, both [^{64}Cu]-AmBaSar-RGD and [^{64}Cu]-DOTA-RGD were shown to bind the U-87MG cells and only 0.1%–0.4% of the radiochemicals was internalized by the cells (3). In addition, the cellular binding of both tracers was shown to be blocked by an excess amount of unlabeled c(RGDyK) (2 μg).

Animal Studies

Rodents

[PubMed]

The metabolic stability of [^{64}Cu]-AmBaSar-RGD and [^{64}Cu]-DOTA-RGD was evaluated in an athymic nude mouse bearing a U-87MG cell tumor as described by Cai et al. (3). The animal was administered either [^{64}Cu]-AmBaSar-RGD or [^{64}Cu]-DOTA-RGD through the intravenous route and euthanized 1 h later for the collection of blood and to obtain the liver, kidneys, and tumors from the animals. Subsequently, the blood and the kidney, liver, and tumor homogenate extracts were prepared and analyzed for uptake of the two radiochemicals with HPLC as detailed elsewhere (3). The amount of intact tracer in the blood, tumor, liver, and kidneys was reported to be 88%, 95%, 98%, and 98%, respectively, for [^{64}Cu]-AmBaSar-RGD and 38%, 87%, 34%, and 74%, respectively, for [^{64}Cu]-DOTA-RGD. This study indicated that [^{64}Cu]-AmBaSar-RGD was more stable than [^{64}Cu]-DOTA-RGD under *in vivo* conditions.

For the biodistribution study, nude mice bearing U-87MG cell tumors (n = 3/time point) were injected with the two tracers respectively and euthanized 20 h later to harvest all the major organs and determine the amount of radioactivity incorporated in the organs (3). Results obtained from this study were presented as percent injected dose per gram tissue (% ID/g), and the tumor/tissue ratios for the various organs were also calculated. At 20 h postinjection (p.i.), both tracers were shown to accumulate primarily in the kidneys, liver, stomach, intestine, and the tumors. With [^{64}Cu]-AmBaSar-RGD, the uptake of radioactivity in the liver and kidneys was 0.55 ± 0.06% ID/g and 1.51 ± 0.27% ID/g, respectively, and for [^{64}Cu]-DOTA-RGD the uptake in the liver and kidneys was 2.16 ± 0.85% ID/g and 0.98 ± 0.40% ID/g, respectively. For [^{64}Cu]-AmBaSar-RGD, the tumor/muscle, tumor/heart, tumor/lung, tumor/liver, and tumor/kidney uptake ratios were 14.20 ± 3.84, 7.33 ± 1.55, 3.34 ± 0.70, 1.18 ± 0.05, and 0.43 ± 0.06, respectively. These ratios were 5.97 ± 2.25, 1.78 ± 0.37, 1.27 ± 0.37, 0.33 ± 0.09, and 0.66 ± 0.04, respectively, for [^{64}Cu]-DOTA-RGD. This study indicated that both tracers had a similar uptake trend in the tissues of the animals.

In another study, the animals (n = 3 mice/tracer) were injected with either [^{64}Cu]-AmBaSar-RGD or [^{64}Cu]-DOTA-RGD in the presence of excess c(RGDyK) (10 mg/kg body weight), and the mice were euthanized 2 h later to determine the amount of radioactivity accumulated in the tumors and the major organ tissues (3). Control mice were injected with the radiochemicals alone and treated as the test animals. A significantly lower ($P < 0.05$) amount of radioactivity (0.09 ± 0.03% ID/g) accumulated in the tumors of animals co-injected with [^{64}Cu]-AmBaSar-RGD and c(RGDyK) compared to tumors of control mice (1.85 ± 0.16% ID/g). A similar trend was noticed with [^{64}Cu]-DOTA-RGD. This study indicated that [^{64}Cu]-AmBaSar-RGD bound specifically to the integrin $\alpha_v\beta_3$ receptors.

Static microPET scans were performed on nude mice bearing U-87MG cell tumors at 1, 2, 4, and 20 h after injection of [^{64}Cu]-AmBaSar-RGD or [^{64}Cu]-DOTA-RGD (n = 3 animals/time point) to determine the biodistribution pattern and tumor-targeting specificity of the tracers in the animals (3). From the PET images it was evident that both tracers accumulated in the tumors to a comparable extent at all time points; however, the biodistribution patterns of the two radiochemicals in the animals were significantly different from each other, especially in the liver and kidneys. The renal uptake of [^{64}Cu]-AmBaSar-RGD reduced from 2.83 ± 0.77% ID/g at 1 h p.i. to 1.43 ± 0.35% ID/g at 20 h p.i., and for [^{64}Cu]-DOTA-RGD the accumulation was 1.52 ± 0.46% ID/g at 1 h p.i. and showed only a slight change at 20 h p.i. (1.58 ± 0.13% ID/g). In general, the accumulation of [^{64}Cu]-AmBaSar-RGD in the kidneys was higher than that of [^{64}Cu]-DOTA-RGD from 1–4 h, and both tracers showed comparable levels of residual label in these organs at 20 h p.i. In the liver, the uptake of [^{64}Cu]-AmBaSar-RGD was 0.89 ± 0.13% ID/g at 1 h p.i. and decreased to 0.64 ± 0.06% ID/g by 20 h p.i. The uptake of [^{64}Cu]-DOTA-RGD in the liver was 2.28 ± 1.08% ID/g at 1 h p.i. and increased slightly to 2.52 ± 1.43% ID/g at 20 h p.i. The investigators noted that the uptake of [^{64}Cu]-DOTA-RGD in the liver was significantly higher (P value not reported) than that of [^{64}Cu]-AmBaSar-RGD at all time points.

MicroPET scans of mice (n = 3 animals/tracer) bearing U-87MG cell tumors injected with either [^{64}Cu]-AmBaSar-RGD or [^{64}Cu]-DOTA-RGD in the presence of a blocking dose of c(RDGyK) showed an almost complete elimination of tracer uptake by the tumors (3). This study confirmed the earlier observations made during the blocking study (described above) that [^{64}Cu]-AmBaSar-RGD specifically bound the integrin $\alpha_v\beta_3$ receptors.

From these studies, the investigators concluded that [^{64}Cu]-AmBaSar-RGD had excellent metabolic stability and favorable *in vivo* pharmacokinetics for the PET detection of tumors expressing integrin $\alpha_v\beta_3$ receptors (3).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

References

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