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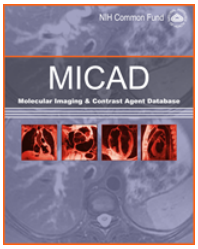

^{68}Ga-1,4,7-Triazacyclononane-1,4-7-triacetic acid-Glu-[15-amino-4,7,10,13-tetraoxapentadecanoic acid-c(Arg-Gly-Asp-D-Phe-Lys)]$_2$

^{68}Ga-NOTA-P$_4$-RGD2

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Chemical name:	^{68}Ga-1,4,7-Triazacyclononane-1,4-7-triacetic acid-Glu-[15-amino-4,7,10,13-tetraoxapentadecanoic acid-c(Arg-Gly-Asp-D-Phe-Lys)]$_2$	
Abbreviated name:	^{68}Ga-NOTA-P$_4$-RGD2, ^{68}Ga-NOTA-E-[PEG$_4$-c(RGDfK)]$_2$	
Synonym:		
Agent category:	Peptide	
Target:	Integrin $\alpha_v\beta_3$	
Target category:	Receptor	
Method of detection:	Positron emission tomography (PET)	
Source of signal\contrast:	^{68}Ga	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$.

Background

[PubMed]

Integrins are a family of heterodimeric glycoproteins on cell surfaces that mediate diverse biological events involving cell–cell and cell–matrix interactions (1). 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 (2-7). 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. The $\alpha_v\beta_3$ antagonists are being studied as antitumor and antiangiogenic agents, and the agonists are being studied as angiogenic agents for coronary angiogenesis (6, 8, 9). The peptide sequence Arg-Gly-Asp (RGD) has been identified as a

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recognition motif used by extracellular matrix proteins (vitronectin, fibrinogen, laminin, and collagen) to bind to a variety of integrins, including $\alpha_v\beta_3$. Various radiolabeled antagonists have been introduced for imaging of tumors and tumor angiogenesis (10).

Most of the cyclic RGD peptides are composed of five amino acids. Various cyclic RGD peptides exhibit selective inhibition of binding to $\alpha_v\beta_3$ (50% inhibition concentration (IC_{50}), 7–40 nM) but not to $\alpha_v\beta_5$ (IC_{50}, 600–4,000 nM) or $\alpha_{IIb}\beta_3$ (IC_{50}, 700–5,000 nM) integrins (11). Various radiolabeled cyclic RGD peptides and peptidominetics have been found to have high accumulation in tumors in mice (12, 13). Out of these developments [^{18}F]Galacto-c(RGDfK) has been evaluated in a number of clinical studies for imaging of $\alpha_v\beta_3$ in cancer patients (14-19). Liu et al. (20) used 1,4,7-triazacyclononane-1,4-7-triacetic acid (NOTA) as a bifunctional chelator for labeling Glu-[15-amino-4,7,10,13-tetraoxapentadecanoic acid-cyclo(RGDfK)]$_2$ (P_4-RGD2) to form (^{68}Ga-NOTA-P_4-RGD2) for positron emission tomography (PET) imaging of $\alpha_v\beta_3$ receptors in nude mice bearing human glioblastoma U87MG tumors.

Synthesis

[PubMed]

P_4-RGD2 was prepared with solid-phase peptide synthesis (20). Addition of the NOTA group to P_4-RGD2 was performed by mixing 2 μmol P_4-RGD2 with 6 μmol *S*-2-(4-isothiocyanatobenzyl)-NOTA in sodium bicarbonate buffer (pH 9) for 5 h at room temperature. NOTA-P_4-RGD2 was isolated with high-performance liquid chromatography (HPLC) with 42% yield and >95% purity. Molecular weight was determined with MALDI-TOF-MS to be *m/z* 2,265.80 Da (calculated molecular weight, 2,263.52 Da). For ^{68}Ga labeling, a solution of 185 MBq (5 mCi) $^{68}GaCl_3$ and 10 nmol RGD2 was heated for 10 min at 42°C. ^{68}Ga-NOTA-P_4-RGD2 was purified with HPLC with a yield of 92% and a radiochemical purity of >98%. The specific activity was 9.8–11.8 MBq/nmol (0.26–0.32 mCi/nmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Liu et al. (20) performed *in vitro* inhibition studies of NOTA-P_4-RGD2 in cultured human U87MG cells with ^{125}I-echistatin. The IC_{50} values were 88.8, 41.8, 100.0, and 34.0 nM for RGD2, P_4-RGD2, NOTA-RGD2, and NOTA-P_4-RGD2, respectively. These comparable IC_{50} values indicated that NOTA and P_4 conjugation had little effect on the receptor binding affinity.

Animal Studies

Rodents

[PubMed]

Liu et al. (20) performed *ex vivo* biodistribution studies of 0.37 MBq (10 μCi) ^{68}Ga-NOTA-P_4-RGD2 or ^{68}Ga-NOTA-RGD2 in nude mice bearing U87MG xenografts at 1 h after injection. Tumor accumulation was 7.98 ± 0.94% and 4.17 ± 1.10% injected dose per gram (ID/g) for ^{68}Ga-NOTA-P_4-RGD2 and ^{68}Ga-NOTA-RGD2, respectively. The tumor/muscle ratios were 6.4 and 2.9 for ^{68}Ga-NOTA-P_4-RGD2 and ^{68}Ga-NOTA-RGD2, respectively. Therefore, the P_4-linkers improved the tumor accumulation. The kidney accumulation was 11.6% ID/g for ^{68}Ga-NOTA-P_4-RGD2 and 11.1% ID/g for ^{68}Ga-NOTA-RGD2. Coinjection of excess c(RGDyK) (10 mg/kg) and ^{68}Ga-NOTA-RGD2 inhibited the tumor accumulation by 80%, whereas the accumulation in the kidneys was inhibited by only 30%. Various small inhibitory effects were also observed in the other tissues and organs. Quantitative analyses of PET imaging data showed that the radioactivity levels in the U87MG tumors (n = 4 mice) were 10.13 ± 1.81% ID/g (30 min), 7.40

± 0.39% ID/g (60 min), and 7.24 ± 0.45% ID/g (120 min) for ^{68}Ga-NOTA-P_4-RGD2. ^{68}Ga-NOTA-P_4-RGD2 was excreted mainly through the kidneys. Coinjection of c(RGDyK) and ^{68}Ga-NOTA-P_4-RGD2 reduced the tumor accumulation to the background level with 81% inhibition at 60 min after injection. The tumor/muscle, tumor/liver, tumor/blood, and tumor/kidney ratios for ^{68}Ga-NOTA-P_4-RGD2 were significantly higher than those for ^{68}Ga-NOTA-RGD2 ($P < 0.01$) at the three time points.

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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