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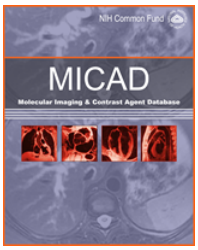

N-[2-(4-[^{18}F]Fluorobenzamido)ethyl]maleimide-sulfhydryl-cyclic-arginine-glycine-aspartic acid peptide

[^{18}F]FBEM-SRGD

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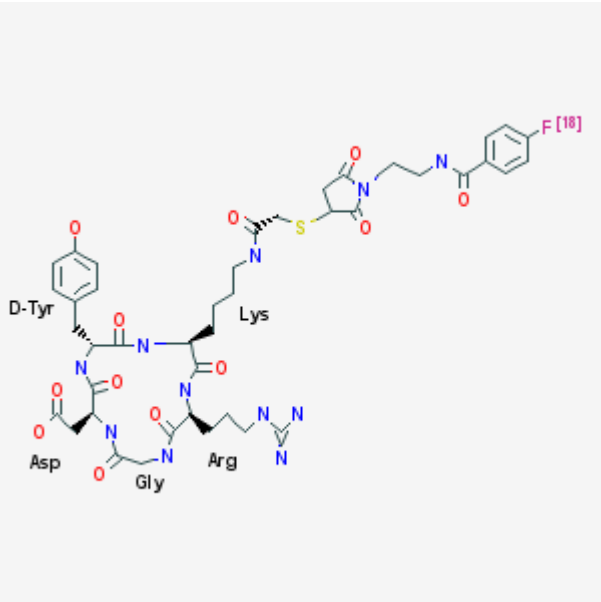
Chemical name:	*N*-[2-(4-[^{18}F]Fluorobenzamido)ethyl]maleimide-sulfhydryl-cyclic-arginine-glycine-aspartic acid peptide
Abbreviated name:	[^{18}F]FBEM-SRGD
Synonym:	^{18}F-RGD
Agent Category:	Peptide
Target:	Integrin $\alpha_v\beta_3$
Target Category:	Receptor binding
Method of detection:	Positron Emission Tomography (PET)
Source of signal:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



Click on the above structure for additional information in PubChem. Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$.

Background

[PubMed]

N-[2-(4-[^{18}F]Fluorobenzamido)ethyl]maleimide-sulfhydryl-cyclic-arginine-glycine-aspartic acid peptide ([^{18}F]FBEM-SRGD) is an integrin-targeted molecular imaging agent developed for positron emission

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tomography (PET) of tumor vasculature and angiogenesis (1). ^{18}F is a positron emitter with a physical half-life ($t_{½}$) of 110 min.

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. It is significantly upregulated in certain types of tumor cells and in almost all tumor vasculature.

Molecular imaging probes carrying the RGD motif that binds to the integrin $\alpha_v\beta_3$ can be used to image tumor vasculature and evaluate angiogenic response to tumor therapy (6, 7). Various RGD peptides in both linear and cyclic forms have been developed for *in vivo* binding to integrin $\alpha_v\beta_3$ (8). Chen et al. (9) evaluated ^{64}Cu-labeled and 18F-labeled cyclic RGD peptide [c(RGDyK)] monomers in nude mice bearing breast tumors. [^{18}F]FB-c(RGDyK) showed high tumor accumulation but also rapid tumor washout with unfavorable biliary excretion (10). To improve the pharmacokinetics and tumor retention of the radiolabeled peptide, a dimer analog was synthesized as [^{18}F]FB-[c(RGDyK)]$_2$, which showed improved tumor localization and predominant renal excretion (11). Radiofluorination can generally be achieved through the functional groups of amino, carboxylic acid, and sulfhydryl. Labeling of RGD peptide with ^{18}F in most fluorinated peptide studies used ^{18}F-synthons such as *N*-succinimidyl 4-[^{18}F]fluorobenzoate to form a stable amide bond by reacting with primary amino groups of RGD peptides at the N terminus or the lysine side chain (1). One of the main concerns with this approach is the potential interference with the biological activities of these peptides. Cai et al. (1) suggested that ^{18}F-labeling of RGD peptides *via* the carboxylic acid group at the C terminus or internal glutamic/aspartic acid side chain might minimize the potential interference. They reported the use of a thiol-reactive ^{18}F-synthon, ^{18}F-FBEM, as the prosthetic group for labeling a sulfhydryl-functionalized c(RGDyK) monomeric peptide (SRGD) and a E[c(RGDyK)]$_2$ dimeric peptide (SRGD2). The maleimide group in ^{18}F-FBEM allows for a thiol-specific Michael addition reaction.

Synthesis

[PubMed]

The c(RGDyK) peptide was first prepared *via* solution cyclization of the fully protected linear pentapeptide H-Gly-Asp(OtBu)-D-Tyr(OtBu)-Lys(Boc)-Arg(Pbf)-OH, followed by deprotection with trifluoroacetic acid (TFA) in the presence of the free radical scavenger triisopropylsilane (1, 12). The c(RGDyK) was conjugated to *N*-succinimidyl *S*-acetylthioacetate (SATA) by mixing c(RGDyK) in sodium borate ($Na_2B_4O_7$) buffer (pH 8.5) with SATA in dimethyl sulfoxide (1). The yield was 95%. The crude SATA-c(RGDyK) was then dissolved in water and mixed with L-hydroxylamine (pH 6.0) for 2 h to yield the SRGD. The final product was purified with semi-preparative high-performance liquid chromatography (HPLC). The overall yield was 80%. The identity of the SRGD peptide ($C_{29}H_{43}N_9O_9S$) was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy. FBEM was obtained by reacting *N*-(2-aminoethyl)maleimide with *N*-succinimidyl 4-fluorobenzoate in $Na_2B_4O_7$ (pH 8.5) at 50°C for 20 min. The reaction was quenched with 2% TFA. The yield was 85% after HPLC purification.

Cai et al. (1) reported the radiolabeling of [^{18}F]FBEM-SRGD with the use of [^{18}F]FBEM, which was first prepared from *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) (10, 13). Then [^{18}F]SFB in acetonitrile was mixed with *N*-(2-aminoethyl)maleimide and *N*,*N*-diisopropylethylamine. The reaction mixture was heated at

40°C for 20 min and then quenched with TFA. The HPLC-purified [^{18}F]FBEM was obtained with a total reaction time of 150 ± 20 min. The non–decay-corrected radiochemical yield was 5 ± 2% (on the basis of ^{18}F-F–) with a specific activity of 150–200 TBq/mmol (4,050–5,400 Ci/mmol). Purified [^{18}F]FBEM in phosphate-buffered saline was mixed with the SRGD peptide in dimethyl sulfoxide and tris(2-carboxyethyl)phosphine hydrochloride in water. The pH of the mixture was adjusted to 7.0–7.5 and incubated at room temperature for 20 min. The resulting [^{18}F]FBEM-SRGD was purified with HPLC. The radiochemical yield was 85 ± 5% (non–decay-corrected) on the basis of [^{18}F]FBEM. The total reaction time, including final HPLC purification, was ~200 ± 25 min. The overall decay-corrected radiochemical yield was 20 ± 4% (n = 5), and the specific activity was 100–150 TBq/mmol (2,700–4,050 Ci/mmol). The radiochemical purity was >98%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cai et al. (1) determined the octanol/water partition coefficient (Log P) of [^{18}F]FBEM-SRGD to be 0.93 ± 0.02. *In vitro* cell-binding affinity studies of the unlabeled FBEM-SRGD was conducted in U87MG glioblastoma cells with ^{125}I-echistatin as the integrin $\alpha_v\beta_3$–specific radioligand. The inhibition concentration (IC_{50}) for FBEM-SRGD was determined to be 66.8 ± 5.1 nM. In comparison, the IC_{50} for c(RGDyK) was determined to be 51.3 ± 4.2 nM.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of [^{18}F]FBEM-SRGD were conducted in nude mice bearing the U87MG glioblastoma on the right front leg and the MDA-MB-435 breast cancer carcinoma on the left mammary fat pad (1). Both tumor volumes reached ~30–400 mm^3. Each mouse received 1 MBq (27 μCi) of [^{18}F]FBEM-SRGD by i.v. administration. At 60 min, the radioactivity levels (n = 3) in percentage of injected dose per gram (% ID/g) detected in the U87MG and MDA-MB-435 tumors were 1.33 ± 0.28 and 1.43 ± 0.11, respectively. In comparison, the radioactivity levels in the U87MG and MDA-MB-435 tumors for the dimeric [^{18}F]FBEM-SRGD2 were 2.71 ± 0.19% ID/g and 5.25 ± 0.17% ID/g, respectively. When blocked by coinjection of 10 mg/kg c(RGDyK), these levels decreased to 0.40 ± 0.02% ID/g ($P < 0.05$) and 0.77 ± 0.04% ID/g ($P < 0.05$), respectively. [^{18}F]FBEM-SRGD was relatively hydrophobic and exhibited high hepatobiliary excretion. By extrapolation from Figure 3 of the article by Cai et al. (1), the radioactivity levels at 10 min were estimated to be ~3% ID/g, ~10% ID/g, ~9% ID/g, and ~2% ID/g for the blood, liver, kidney, and intestine, respectively. At 60 min, these levels appeared to change to ~0.2% ID/g, ~0.5% ID/g, ~1% ID/g, and ~10% ID/g, respectively. Blocking with c(RGDyK) increased the intestinal radioactivity level to 16.57 ± 0.81% ID/g at 60 min. MicroPET imaging of 3.7 MBq (100 μCi) [^{18}F]FBEM-SRGD was performed in nude mice bearing both the s.c. U87MG tumor and the orthotopic MDA-MB-435 tumor (1). The radioligand appeared to be excreted by both the liver and kidneys. There was also a fair amount of radioactivity in the abdomen up to 4 h. High radioactivity accumulation was observed in the tumor as early as 6 min. The liver and kidney radioactivity levels appeared to be much higher than that of the tumors.

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

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