[^{18}F]FB-NH-mini-PEG-E{E[c(RGDyK)]$_2$}$_2$

[^{18}F]FPRGD4

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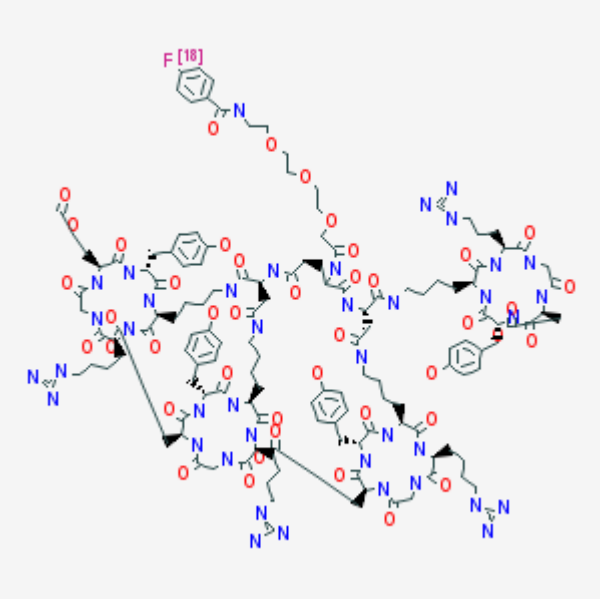
Chemical name:	[^{18}F]FB-NH-mini-PEG-E{E(c(RGDyK)]$_2$}$_2$
Abbreviated name:	[^{18}F]FPRGD4
Synonym:	^{18}F-PEG-RGD, ^{18}F-labeled PEGylated tetrameric RGD peptide
Agent Category:	Peptide
Target:	Integrin $\alpha_v\beta_3$
Target Category:	Receptor binding
Method of detection:	Positron Emission Tomography (PET)
Source of signal / contrast:	^{18}F
Activation:	No
Studies:	• *In vitro* • Rodents



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

Background

[PubMed]

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[^{18}F]FB-NH-mini-PEG-E{E[c(RGDyK)]$_2$}$_2$ ([^{18}F]FPRGD4) is an integrin-targeted molecular imaging agent developed for positron emission 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 physiologic 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 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 (RGDfK or RGDyK) have been developed for *in vivo* binding to integrin $\alpha_v\beta_3$ (8). 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 (9). Alternatively, Chen et al. (10) modified c(RGDyK) with monofunctional methoxy-polyethylene glycol (mPEG; molecular weight = 2,000 kDa) 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. (11) inserted a heterofunctional PEG (molecular weight = 3,400 kDa) molecule between the [^{18}F]fluorobenzoyl component and the RGD peptide to produce [^{18}F]FB-PEG-c(RGDyK) for imaging of brain tumor angiogenesis. The PEGylated [^{18}F]FB-c(RGDyK) analog appeared to improve tumor retention and *in vivo* kinetics compared with [^{18}F]FB-c(RGDyK). These improvements might be attributed to a number of possible causes that include shielding of antigenic and immunogenic epitopes, shielding receptor-mediated uptake by the reticuloendothelial systems, preventing the recognition and degradation by proteolytic enzymes, and increasing the apparent size of the peptide. Wu et al. (12) reported that a multimeric RGD peptide with more than two repeating cyclic RGD units would further enhance the affinity of the receptor–ligand interactions through the phenomenon of a polyvalency effect. However, the attempt of synthesis of 18F-labeled tetrameric RGDyK peptiode (^{18}F-FRGD4) proved to be difficult and gave very low yield (<2%) because of increased molecular size and steric hindrance (1). On the basis of these observations, Wu et al. (1) inserted a mini-PEG linker to improve the labeling yield and successfully prepared [^{18}F]FPRGD4 by radiolabeling PEGylated tetrameric RGD peptide

with reasonable yield. The *in vivo* kinetics of this PEGylated probe was studied in three different tumor models.

Synthesis

[PubMed]

Wu et al. (12) reported the preparation of cyclic RGD peptide tetramer E{E[c(RGDyK)]$_2$}$_2$ (RGD4) from the cyclic RGD dimer E[c(RGDyK)]$_2$. RGD4 was then used to prepare NH_2-Mini-PEG-E{E[c(RGDyK)]$_2$}$_2$ (PRGD4) (1). Briefly, Boc-11-amino-3,6,9-trioxaundecanoic acid (Boc-NH-mini-PEG-OOH) was mixed with *N*,*N*-diisopropylethylamine and *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyl-uronium tetrafluoroborate (1). The reaction mixture was stirred at room temperature for 0.5 h. RGD4 in *N*,*N*-dimethylformamide was added to the mixture and stirred at room temperature for 2 h to produce Boc-NH-mini-PEG-E{E[c(RGDyK)]$_2$}$_2$. The yield was 60%. The Boc-group was then removed by treatment with trifluoroacetic acid (TFA) for 5 min at room temperature to yield PRGD4. PRGD4 was purified by high-performance liquid chromatography (HPLC) for radiolabeling. The identify of PRGD4 was confirmed by matrix-assisted laser desorption/ionization mass spectrometry with m/z 3,001.0 for $[MH]^+$ (the calculated molecular weight of $C_{131}H_{194}N_{40}O_{42}$ = 3,001.1).

The purified product of PRGD4 was labeled with ^{18}F by coupling with *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB) (1, 13). The synthesis was conducted with a commercial synthesis module. The total synthesis time was ~100 min and the decay-corrected yield was 67 ± 11% (n = 10). The purified [^{18}F]SFB was added to PRGD4 in dimethyl sulfoxide with DIPEA. This peptide mixture was incubated at 60°C for 30 min. The final product was then diluted with 1% TFA in water before purified by semipreparative HPLC to yield [^{18}F]FPRGD4. The decay-corrected radiochemical yield based on [^{18}F]SFB was 22.0 ± 0.8% (n = 4). Analytical HPLC showed that the radiochemical purity was >99%. The specific activity was ~100–200 GBq/μmol (2.7-5.4 Ci/μmol) based on [^{18}F]SFB. Starting from ^{18}F-F^-, the total synthesis time of [^{18}F]FPRGD4 was ~180 min (including HPLC purification), and the overall decay-corrected yield was 15 ± 4%.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The octanol-water partition coefficient (log P) of [^{18}F]FPRGD4 was determined to be –2.67 ± 0.22 with the use of phosphate-buffered saline and octanol (1). Wu et al. (1) conducted an *in vitro* cell (U87MG human glioblastoma) integrin receptor-binding assays with [^{18}F]FPRGD4 by using ^{125}I-echistatin (integrin $\alpha_v\beta_3$ specific) in displacement studies. The 50% inhibitory concentration IC_{50} for [^{18}F]FPRGD4 was 37.7 ± 7.0 nM (n = 3). In comparison, the IC_{50} values for RGD4 and PRGD4 were 39.1 ± 5.5 and 46.5 ± 5.3, respectively.

Animal Studies

Rodents

[PubMed]

MicroPET imaging of 3.7 MBq (100 μCi) [^{18}F]FPRGD4 in nude mice (n = 3) bearing the s.c. U87MG tumor (100-300 mm^3) as early as 5 min clearly visualized the tumor with a high contrast to contralateral background (1). The radiolabeled peptide was cleared mainly through the kidneys with some hepatic clearance. Using the region-of-interest (ROI) technique, the tumor radioactivity levels (% ID/g) were calculated to be 9.87 ± 0.10 (5 min), 7.80 ± 0.14 (30 min), 6.40 ± 0.27 (60 min), 5.39 ± 0.14 (120 min), and 4.82 ± 0.22 (180 min). When a blocking dose of 10 mg/kg c(RGDyK) was given, more than 80% of the tumor radioactivity was inhibited. Other major organ radioactivity levels were also reduced.

MicroPET imaging of 3.7 MBq (100 μCi) [^{18}F]FPRGD4 was conducted in nude mice (n = 3) bearing the MDA-MB-435 tumor (orthotopic injection into the left mammary fat pad) which had lower integrin expression level than the U87MG tumor (1). The ROI tumor radioactivity levels (% ID/g) were lower that those of U87MG model with 5.07 ± 0.18 (30 min), 4.53 ± 0.36 (60 min), and 3.38 ± 0.48 (150 min). MicroPET imaging of 3.7 MBq (100 μCi) [^{18}F]FPRGD4 was also performed in a spontaneous murine mammary carcinoma model grown in c-neu onomice (n = 3) (1). The ROI tumor radioactivity levels (% ID/g) were lower that those of U87MG model with 4.22 ± 0.18 (30 min), 3.56 ± 0.36 (60 min), and 2.36 ± 0.40 (150 min). Immunofluorescence staining of frozen tissue slices from c-neu oncomice confirmed the expression of β_3-integrin in both tumor cells and endothelial cells of the murine mammary carcinoma (1). β_3-integrin was also detected in the liver, lung, kidneys, and glomerulus. No significant difference in organ distribution of [^{18}F]FPRGD4 was observed among all three animal models. In a control study, microPET imaging of 3.7 MBq (100 μCi) [^{18}F]FPRGD4 in nude mice (n = 3) bearing an integrin-negative s.c. DU145 tumor. The ROI tumor radioactivity levels (%ID/g) were significantly lower with 1.44 ± 0.34 (30 min) and 0.93 ± 0.13 (60 min) (1).

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

NIH NIBIB R21 EB001785, NCI CA102123, P50 CA114747, U54 CA119367, R24 CA93862.

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