



^{99m}Tc -Hydrazinonicotinyl(tricine)(TPPTS)-Glu-c(RGDyK)-bombesin[7-14]NH₂

^{99m}Tc -HYNIC-RGD-BBN

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Chemical name:	^{99m}Tc - Hydrazinonicotinyl(tricine)(TPPTS)-Glu-c(RGDyK)-bombesin[7-14]NH ₂	
Abbreviated name:	^{99m}Tc -HYNIC-RGD-BBN	
Synonym:		
Agent category:	Peptide	
Target:	Gastrin-releasing peptide receptor (GRPR), integrin $\alpha_v\beta_3$	
Target category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT), gamma planar imaging	
Source of signal\contrast:	^{99m}Tc	
Activation:	No	
Studies:	<ul style="list-style-type: none"> <i>In vitro</i> Rodents 	Click on protein , nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$.

Background

[PubMed]

The amphibian bombesin (BBN or BN, a peptide of 14 amino acids) is an analog of human gastrin-releasing peptide (GRP, a peptide of 27 amino acids) that binds to GRP receptors (GRPR) with high affinity and specificity (1, 2). Both GRP and BBN share an amidated C-terminus sequence homology of seven amino acids, Trp-Ala-Val-Gly-His-Leu-Met-NH₂. BBN-Like peptides have been shown to induce various biological responses in diverse tissues, including the central nervous system (CNS) and the gastrointestinal (GI) system. They also act as potential growth factors for both normal and neoplastic tissues (3). Specific BBN receptors (BBN-Rs) have been identified on CNS and GI tissues and on a number of tumor cell lines (4). The BBN-R superfamily includes at least four different subtypes, namely the GRPR subtype (BB2), the neuromedin B receptor subtype (BB1), the BB3 subtype, and the BB4 subtype. The findings of GRPR overexpression in various human tumors, such as

breast, prostate, lung, colon, ovarian, and pancreatic cancers, provide opportunities for tumor imaging by designing specific molecular imaging agents to target the GRPR (5, 6).

Integrins are a family of heterodimeric glycoproteins on cell surfaces that mediate diverse biological events involving cell–cell and cell–matrix interactions (7). 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 (8-13). 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. A peptide sequence consisting of Arg-Gly-Asp (RGD) has been identified as a 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 ligands have been introduced for imaging of tumors and tumor angiogenesis (14).

Because breast and prostate cancers express both GRPR and $\alpha_v\beta_3$, Liu et al. (15) designed an RGD-BBN heterodimer in which BBN[7-14]NH₂ and c(RGDyK) are connected with a glutamate linker (BBN on the Glu side chain γ -carboxylate group and RGD on the Glu side chain α -carboxylate group). 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) was used as a bifunctional chelator for labeling RGD-BBN to form ⁶⁴Cu-NOTA-RGD-BBN for use in positron emission tomography (PET) imaging of $\alpha_v\beta_3$ and GRPR in nude mice bearing human tumors. For single-photon emission computed tomography (SPECT), Liu et al. (16) conjugated Glu-RGD-BBN with 6-hydrazinonicotinyl (HYNIC) and labeled the product with ^{99m}Tc, using tricine and trisodium triphenylphosphine-trisulfonate (TPPTS) as the coligands. ^{99m}Tc-HYNIC-RGD-BBN was evaluated in C57/BL6 mice bearing mouse Lewis lung carcinomas (LLC).

Related Resource Links:

- Chapters in MICAD ([GRPR](#), [RGD](#))
- Gene information in NCBI ([GRPR](#), [GRP](#), [\$\alpha_v\$ integrin](#), [\$\beta_3\$ integrin](#))
- Articles in Online Mendelian Inheritance in Man (OMIM) ([GRPR](#), [GRP](#), [\$\alpha_v\$ integrin](#), [\$\beta_3\$ integrin](#))
- Clinical trials ([GRPR](#), [RGD](#))
- Drug information in FDA ([RGD](#))

Synthesis

[PubMed]

RGD-BBN was prepared with solid-phase peptide synthesis (16). Addition of a HYNIC group to RGD-BBN was performed by mixing 2 μ mol RGD-BBN with 6 μ mol HYNIC-NHS in sodium bicarbonate buffer (pH 9) for 5 h at room temperature. HYNIC-RGD-BBN was isolated with high-performance liquid chromatography (HPLC), with 56% yield. Measurement with matrix-assisted laser desorption ionization/time of flight mass spectrometry indicated the molecular mass to be m/z 1,918.60 (calculated molecular weight, 1,919.17). For ^{99m}Tc labeling, a solution of 370 MBq (10 mCi) Na^{99m}TcO₄, SnCl₂, and 10 nmol HYNIC-RGD-BBN in succinate buffer (pH 5) was incubated for 10 min at 25°C. After addition of TPPTS, the mixture was heated for 30 min at 100°C. ^{99m}Tc-HYNIC-RGD-BBN was isolated with HPLC, with 90% yield and a radiochemical purity of >98%. The specific activity was >30 MBq/nmol (0.81 mCi/nmol). The total preparation time was ~50 min.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Liu et al. (16) performed *in vitro* inhibition studies of HYNIC-RGD-BBN in cultured U87MG cells with ¹²⁵I-c(RGDyK). The 50% inhibition concentration (IC₅₀) values were 18.8 \pm 3.8 nM and 10.8 \pm 2.6 nM for HYNIC-RGD-BBN and c(RGDyK), respectively. *In vitro* inhibition studies of HYNIC-RGD-BBN were also performed in

cultured PC-3 cells (high GRPR and moderate $\alpha_v\beta_3$ levels) with ^{125}I -[Tyr⁴]-BBN; the IC₅₀ values were 104.7 ± 5.8 nM and 71.6 ± 3.1 nM for HYNIC-RGD-BBN and BBN, respectively. The HYNIC-RGD-BBN heterodimer exhibited slightly lower binding affinities for the GRPR and $\alpha_v\beta_3$ integrin receptor than the corresponding unconjugated RGD and BBN peptides. Cellular accumulation of ^{99m}Tc -HYNIC-RGD-BBN in PC-3 tumor cells showed a gradual increase in radioactivity in PC-3 cells from 30 min to 4 h. The cellular radioactivity reached 4.0% of incubation dose at 4 h. Treatment with excess RGD-BBN (1,000 nM) almost completely inhibited the accumulation of ^{99m}Tc -HYNIC-RGD-BBN in PC-3 tumor cells.

Animal Studies

Rodents

[PubMed]

Gamma planar imaging scans were performed in mice ($n = 4/\text{group}$) bearing LLC at 1 h after intravenous injection of 14.8 MBq (400 μCi) ^{99m}Tc -HYNIC-RGD-BBN (16). The tumors and kidneys were clearly visualized. Blocking studies were performed with coinjection of BBN (15 mg/kg), cRGDyK (10 mg/kg), or BBN (15 mg/kg) plus cRGDyK (10 mg/kg). Tumor accumulation of ^{99m}Tc -HYNIC-RGD-BBN exhibited little change with BBN, whereas cRGDyK or BBN plus cRGDyK completely blocked the radioactivity in the tumors. Gamma planar imaging scans were performed in mice ($n = 4/\text{group}$) bearing LLC and inflammation at 1 h after injection of ^{99m}Tc -HYNIC-RGD-BBN or [¹⁸F]Fluoro-2-deoxy-2-D-glucose ([¹⁸F]FDG). High radioactivity levels were observed with FDG in both inflammation sites and LLC tumors, whereas only LLC tumors were visualized with ^{99m}Tc -HYNIC-RGD-BBN. Whole-body SPECT/CT imaging studies were also performed in mice ($n = 4$) bearing LLC at 1.5 h and 3 h after intravenous injection of 37 MBq (1 mCi) ^{99m}Tc -HYNIC-RGD-BBN. Predominant kidney, tumor, and pancreas accumulation of ^{99m}Tc -HYNIC-RGD-BBN was clearly visualized. The pulmonary metastatic lesions were clearly visualized and verified by anatomical examination and histostaining.

Liu et al. (16) performed *ex vivo* biodistribution studies in mice ($n = 4/\text{group}$) bearing LLC at 1 h and 2 h after injection of 0.37 MBq (0.01 mCi) ^{99m}Tc -HYNIC-RGD-BBN. Tumor accumulation of ^{99m}Tc -HYNIC-RGD-BBN was $2.69 \pm 0.66\%$ injected dose/gram (ID/g) at 1 h and $1.99 \pm 0.61\%$ ID/g at 2 h after injection. The inflammation accumulation of ^{99m}Tc -HYNIC-RGD-BBN was $1.20 \pm 0.32\%$ ID/g at 1 h after injection and $0.56 \pm 0.17\%$ ID/g at 2 h after injection. The organ with the highest accumulation at 1 h after injection was the pancreas (26% ID/g), followed by the kidney (17% ID/g), intestine (10% ID/g), and stomach (5% ID/g). The liver, heart, bone, blood, and muscle exhibited <1% ID/g. Co-injection with excess RGD-BBN blocked radioactivity in the pancreas, intestine, stomach, and tumor, whereas little inhibition was observed in the inflammation. [¹⁸F]FDG showed high heart (23.5% ID/g) and kidney (36.0% ID/g) accumulation of radioactivity. The tumor and inflammation accumulation of [¹⁸F]FDG was $6.56 \pm 2.27\%$ ID/g and $5.94 \pm 2.35\%$ ID/g, respectively. The tumor/inflammation and tumor/muscle ratios of [¹⁸F]FDG were >one-fold lower than those of ^{99m}Tc -HYNIC-RGD-BBN ($P < 0.05$).

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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