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Comparison of biological properties of ^{99m}Tc-labeled cyclic RGD Peptide trimer and dimer useful as SPECT radiotracers for tumor imaging

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ABSTRACT

Introduction: This study sought to evaluate a ^{99m}Tc-labeled trimeric cyclic RGD peptide (^{99m}Tc-4P-RGD$_3$) as the new radiotracer for tumor imaging. The objective was to compare its biological properties with those of ^{99m}Tc-3P-RGD$_2$ in the same animal model.
Methods: HYNIC-4P-RGD$_3$ was prepared by reacting 4P-RGD$_3$ with excess HYNIC-OSu in the presence of diisopropylethylamine. ^{99m}Tc-4P-RGD$_3$ was prepared using a kit formulation, and evaluated for its tumor-targeting capability and biodistribution properties in the BALB/c nude mice with U87MG human glioma xenografts. Planar and SPECT imaging studies were performed in athymic nude mice with U87MG glioma xenografts. For comparison purpose, ^{99m}Tc-3P-RGD$_2$ (a $\alpha_v\beta_3$-targeted radiotracer currently under clinical evaluation for tumor imaging in cancer patients) was also evaluated in the same animal models. Blocking experiments were used to demonstrate the $\alpha_v\beta_3$ specificity of ^{99m}Tc-4P-RGD$_3$.
Results: ^{99m}Tc-4P-RGD$_3$ was prepared with >95% RCP and high specific activity (~200 GBq/μmol). ^{99m}Tc-4P-RGD$_3$ and ^{99m}Tc-3P-RGD$_2$ shared almost identical tumor uptake and similar biodistribution properties. ^{99m}Tc-4P-RGD$_3$ had higher uptake than ^{99m}Tc-3P-RGD$_2$ in the intestines and kidneys; but it showed better metabolic stability. The U87MG tumors were clearly visualized by SPECT with excellent contrast with ^{99m}Tc-4P-RGD$_3$ and ^{99m}Tc-3P-RGD$_2$.
Conclusion: Increasing peptide multiplicity from 3P-RGD$_2$ to 4P-RGD$_3$ offers no advantages with respect to the tumor-targeting capability. ^{99m}Tc-4P-RGD$_3$ is as good a SPECT radiotracer as ^{99m}Tc-3P-RGD$_2$ for imaging $\alpha_v\beta_3$-positive tumors.

1. Introduction

Integrin $\alpha_v\beta_3$ is a receptor for the extracellular matrix proteins (e.g. collagen, fibrinogen, fibronectin, laminin and vitronectin) with one or more arginine-glycine-aspartic (RGD) tripeptide sequences. The $\alpha_v\beta_3$ is generally expressed at low levels on epithelial cells and mature endothelial cells, but it is overexpressed on the tumor cells and activated endothelial cells of neovasculature. Because of the role of $\alpha_v\beta_3$ in tumor angiogenesis and metastasis, cyclic RGD peptides are often used as $\alpha_v\beta_3$ antagonists for tumor therapy, and radiolabeled cyclic RGD peptides are utilized as "$\alpha_v\beta_3$–targeted" radiotracers for tumor imaging [1–10]. Over the last several years, we have been interested in radiolabeled multimeric cyclic RGD peptides as radiotracers for imaging $\alpha_v\beta_3$-positive tumors and related metastasis [11–29]. Multiple cyclic RGD moieties are utilized to maximize their $\alpha_v\beta_3$ binding affinity and tumor uptake of their corresponding radiotracers regardless of the attached isotope. We found that there are two important factors (bivalency and locally enhanced RGD concentration) contributing to the higher $\alpha_v\beta_3$ binding affinity of multimeric cyclic RGD peptides than their monomeric analogs [1,20,23]. The concentration factor exists

Abbreviations: HYNIC-OSu, sodium succinimidyl 6-(2-(2-sulfonatobenzaldehyde)hydrazono)nicotinate); ITLC, instant thin layer chromatography; MALDI, matrix-assisted laser desorption ionization; PET, positron emission tomography; RCP, radiochemical purity; SPECT, single photon emission computed tomography; RGD$_2$, E[c(RGDfK)]$_2$ = Glu[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$; RGD$_4$, E{E[c(RGDfK)]$_2$}$_2$ = Glu{E[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$}$_2$; 2P-RGD$_2$, E[PEG$_4$-c(RGDfK)]$_2$ = Glu[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_2$ (PEG$_4$ = 15-amino-4,7,10,13-tetraoxapentadecanoic acid); 3P-RGD$_2$, PEG$_4$-E[PEG$_4$-c(RGKfD)]$_2$ = PEG$_4$-Glu[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_2$; 4P-RGD$_3$, PEG$_4$-ACHDA[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_3$ (ACHDA = 4-amino-4-(2-carboxyethyl)heptanedioic acid); HYNIC-4P-RGD$_3$, HYNIC-PEG$_4$-ACHDA[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG$_4$)]]$_3$ (HYNIC = 6-(2-(2-sulfonatobenzaldehyde)hydrazono)nicotinyl); ^{18}F-Alfatide-II, [^{18}F]AlF(NOTA-2P-RGD$_2$) (NOTA = 1,4,7-tritazacyclononane-1,4,7-triacetic acid); ^{99m}Tc-3P-RGD$_2$, [^{99m}Tc(HYNIC-3P-RGD$_2$)(tricine)(TPPTS)] (TPPTS = trisodium triphenylphosphine-3,3′,3″-trisulfonate); ^{99m}Tc-4P-RGD$_3$, [^{99m}Tc(HYNIC-4P-RGD$_3$)(tricine)(TPPTS)].

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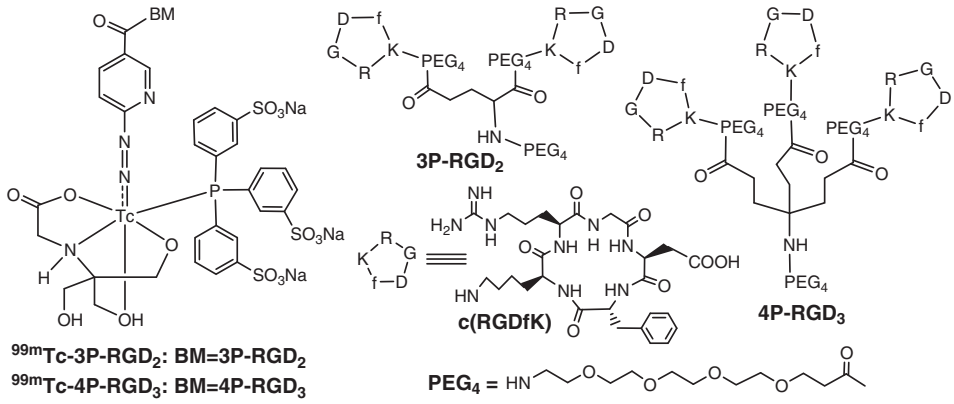

Fig. 1. Chemdraw structures of multimeric cyclic RGD peptides (3P-RGD_2 and 4P-RGD_3) and their ternary ligand complexes [^{99m}Tc(HYNIC-BM)(tricine)(TPPTS)] (BM = biomolecule; ^{99m}Tc-3P-RGD_2: BM = 3P-RGD_2; and ^{99m}Tc-4P-RGD_3: BM = 4P-RGD_3). ^{99m}Tc-4P-RGD_3 is the new SPECT radiotracer evaluated in this study. ^{99m}Tc-3P-RGD_2 is currently under intensive clinical evaluation for tumor imaging in cancer patients [31–35]. It was used for comparison purposes to study the impact of peptide multiplicity on tumor uptake and biodistribution properties in the same tumor-bearing animal model.

in all multimeric RGD peptides regardless of the linker length. The key to achieve bivalency is the distance between two cyclic RGD motifs. We also found that cyclic RGD tetramers (such as RGD_4) are actually bivalent in binding to the $\alpha_v\beta_3$ even though they have four identical cyclic c(RGDfK) motifs [20,23]. Among many of radiolabeled multimeric cyclic peptides, ^{99m}Tc-3P-RGD_2 (Fig. 1) and ^{18}F-Alfatide-II are currently under clinical evaluations as new radiotracers for tumor imaging in cancer patients [30–36]. Since ^{99m}Tc-3P-RGD_2 could be prepared in >95% radiochemical purity, it offers significant advantages over ^{18}F-Alfatide-II, which often requires post-labeling chromatographic purification [35,36]. ^{99m}Tc-3P-RGD_2 SPECT/CT has been used to quantify the tumor uptake [37–40], and to monitor the tumor growth [37,38], the progression of breast cancer lung metastases [39], and the pharmacological effects of antiangiogenic therapy [38,40].

In the literature, only a few radiolabeled cyclic RGD trimers were reported [41,42]. There was no comparison in biodistribution properties between the radiolabeled cyclic RGD dimers and trimers. With this in mind, we designed a new RGD peptide trimer (Fig. 1: 4P-RGD_3 = PEG_4-ACHDA[cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG_4)]]$_3$; and ACHDA = 4-amino-4-(2-carboxyethyl)heptanedioic acid). ACHDA was used to bridge three c(RGDfK) moieties. Four PEG_4 linkers were used to enhance the hydrophilicity of ^{99m}Tc radiotracer and increase the distance between two neighboring c(RGDfK) moieties in 4P-RGD_3 so that it is able to achieve bivalency. In this report, we present the synthesis and biological evaluation of ^{99m}Tc-4P-RGD_3 (Fig. 1) as a new SPECT radiotracer for tumor imaging. ^{99m}Tc-4P-RGD_3 is the first ^{99m}Tc-labeled cyclic RGD peptide trimer. The main objective of this study was to compare the biodistribution and imaging properties of ^{99m}Tc-4P-RGD_3 and ^{99m}Tc-3P-RGD_2 in the same tumor-bearing animal model.

2. Experimental Section

2.1. Materials and Instruments

Tricine and trisodium triphenylphosphine-3,3′,3″-trisulfonate (TPPTS) were purchased from *Sigma/Aldrich* (St. Louis, MO), and were used without further purification. Cyclic RGD peptides RGD_2 (E[c(RGDfK)]$_2$ = Glu[cyclo(Arg-Gly-Asp-D-Phe-Lys)]$_2$) and 4P-RGD_3 (PEG_4-ACHDA{cyclo[Arg-Gly-Asp-D-Phe-Lys(PEG_4)]}$_3$) were custom-made by the Peptides International, Inc. (Louisville, KY). Sodium succinimidyl 6-(2-(2-sulfonatobenzaldehyde)hydrazono)nicotinate (HYNIC-OSu) was prepared according to literature method [43]. HYNIC-3P-RGD_2 and ^{99m}Tc-3P-RGD_2 were prepared using the procedure described in our previous report [14]. Na$^{99m}TcO_4$ was obtained from Cardinal HealthCare® (Indianapolis, IN). The MALDI (matrix-assisted laser desorption ionization) mass spectral data for HYNIC-4P-RGD_3 were collected on an Applied Biosystems Voyager DE PRO mass spectrometer (Framingham, MA), the Department of Chemistry, Purdue University.

2.2. HPLC Methods

HPLC Method 1 used a LabAlliance HPLC system (Scientific Systems, Inc., State College, PA) equipped with a UV/vis detector (λ = 254 nm) and Zorbax C_{18} column (9.4 mm × 250 mm, 100 Å pore size; Agilent Technologies, Santa Clara, CA). The flow rate was 2.5 mL/min with a mobile phase being 90% A and 10% B at 0 min to 80%A and 20% B at 5 min, and to 50%A and 50% B at 20 min. The radio-HPLC (Method 2) used an Agilent HP-1100 HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a β-ram IN/US detector (Tampa, FL) and Zorbax C_8 column (4.6 mm × 250 mm, 300 Å pore size; Agilent Technologies, Santa Clara, CA). The flow rate was 1 mL/min. The mobile phase was isocratic for the first 5 min with 90% A (25 mM NH_4OAc, pH = 6.8) and 10% B (acetonitrile), followed by a gradient mobile phase going from 90% A and 10% B at 5 min to 40% A and 60% B at 20 min. The radiochemical purity was reported as the percentage of area for the peak at 15–16 min on each radio-HPLC chromatogram of ^{99m}Tc-3P-RGD_2 and ^{99m}Tc-4P-RGD_3. The instant thin layer chromatography (ITLC) used Gelman Sciences silica-gel strips and a 1:1 mixture of acetone and saline as the mobile phase. ^{99m}Tc-3P-RGD_2, ^{99m}Tc-4P-RGD_3 and $^{99m}TcO_4^-$ migrated to solvent front while [^{99m}Tc]colloid stayed at the origin. [^{99m}Tc]colloid was reported as the percentage of radioactivity at the origin over the total radioactivity on each strip.

2.3. HYNIC-4P-RGD_3

HYNIC-OSu (13.5 mg, 30 μmol) and 4P-RGD_3 (9.0 mg, 3 μmol) were dissolved in DMF (2 mL). After addition of excess DIEA (5 drops), the mixture was stirred at room temperature for 24 h. To the mixture was added 2 mL of water after completion of the reaction. The pH value was then adjusted to 3–4 using neat TFA. The product was separated from the reaction mixture by HPLC. Fractions at ~18 min were collected. Lyophilization of collected fractions afforded the expected product HYNIC-4P-RGD_3 as a white powder. The yield was 6.5 mg (~50%). MALDI-MS: m/z = 3295.8240 for $[M + H]^+$ (M = 3294.61 calcd. For $[C_{148}H_{227}N_{35}O_{48}S]$).

2.4. ^{99m}Tc-Labeling

To a lyophilized vial containing 20–25 μg HYNIC-RGD conjugate, 5 mg TPPTS, 6.5 mg tricine, 40 mg mannitol, 38.5 mg disodium succinate hexahydrate and 12.7 mg succinic acid was added 1.0–1.5 mL of