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Biodistribution of ^{99m}Tc Labeled Integrin Antagonist

Beom-Su Jang[1,4], Seung-Hee Park[4], In Soo Shin[2], Jin-Soo Maeng[3] and Chang H. Paik[4]

[1]*RI-Biomics Research & Development Team, Korea Atomic Energy Research Institute, Jeonbuk*
[2]*Division of Biologics Research, Korea Food and Drug Administration, Osong, Chungbuk*
[3]*Bio-nanotechnology Research Center, Korea Food Research Institute, Sungnam, Korea*
[4]*Nuclear Medicine Department and Radiology Department, Warren G. Magnuson Clinical Center, NIH, Bethesda, MD 20892, USA*

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The selective targeting of an integrin $\alpha_v\beta_3$ receptor using radioligands may enable the assessment of angiogenesis and integrin $\alpha_v\beta_3$ receptor status in tumors. The aim of this research was to label a peptidomimetic integrin $\alpha_v\beta_3$ antagonist (PIA) with $^{99m}Tc(CO)_3$ and to test its receptor targeting properties in nude mice bearing receptor-positive tumors. PIA was reacted with *tris*-succinimidyl aminotriacetate (TSAT) (20 mM) as a PIA per TSAT. The product, PIA-aminodiacetic acid (ADA), was radiolabeled with $[^{99m}Tc(CO)_3(H_2O)_3]^{+1}$, and purified sequentially on a Sep-Pak C-18 cartridge followed by a Sep-Pak QMA anion exchange cartridge. Using gradient C-18 reverse-phase HPLC, the radiochemical purity of $^{99m}Tc(CO)_3$-ADA-PIA (retention time, 10.5 min) was confirmed to be > 95%. Biodistribution analysis was performed in nude mice (n = 5 per time point) bearing receptor-positive M21 human melanoma xenografts. The mice were administered $^{99m}Tc(CO)_3$-ADA-PIA intravenously. The animals were euthanized at 0.33, 1, and 2 hr after injection for the biodistribution study. A separate group of mice were also co-injected with 200 μg of PIA and euthanized at 1 hr to quantify tumor uptake. $^{99m}Tc(CO)_3$-ADA-PIA was stable in phosphate buffer for 21 hr, but at 3 and 6 hr, 7.9 and 11.5% of the radioactivity was lost as histidine, respectively. In tumor bearing mice, $^{99m}Tc(CO)_3$-ADA-PIA accumulated rapidly in a receptor-positive tumor with a peak uptake at 20 min, and rapid clearance from blood occurring primarily through the hepatobiliary system. At 20 min, the tumor-to-blood ratio was 1.8. At 1 hr, the tumor uptake was 0.47% injected dose (ID)/g, but decreased to 0.12% ID/g when co-injected with an excess amount of PIA, indicating that accumulation was receptor mediated. These results demonstrate successful ^{99m}Tc labeling of a peptidomimetic integrin antagonist that accumulated in a tumor via receptor-specific binding. However, tumor uptake was very low because of low blood concentrations that likely resulted from rapid uptake of the agent into the hepatobiliary system. This study suggests that for $^{99m}Tc(CO)_3$-ADA-PIA to be useful as a tumor detection agent, it will be necessary to improve receptor binding affinity and increase the hydrophilicity of the product to minimize rapid hepatobiliary uptake.

Key words: Peptidomimetic integrin $\alpha_v\beta_3$ antagonist, ^{99m}Tc tricarbonyl precursor, Biodistribution

INTRODUCTION

Integrin $\alpha_v\beta_3$ is a heterodimeric transmembrane glycoprotein and is overexpressed in tumor induced angiogenic vessels and in various malignant human tumors. The selective targeting of this receptor with radioligands may enable an assessment of the angiogenesis and receptor status in tumors. Arginylglycylaspartic acid (RGD) peptides and peptidomimetic antagonists specific for a $\alpha_v\beta_3$ receptor have recently been labeled with various gamma and positron emitters for scintigraphic detection and beta emitters for the radiotherapy of tumors (1-8). Steady progress has been reported in optimizing the labeling methodologies for an increase in tumor to non-tumor tissue ratios, especially a tumor to liver ratio by increasing the hydrophilicity of the product through glycosylation and PEGylation (9-12).

In this research, a peptidomimetic integrin $\alpha_v\beta_3$ antagonist, 4-[2-(3,4,5,6-tetrahydropyrimidine-2-ylamino)ethyloxy]benzoyl-2-(S)-aminoethylsulfonyl-amino-β-alanine (PIA), as an integrin-receptor mediated tumor targeting agent was studied. PIA was previously coupled to a cationic polymerized

Correspondence to: Beom-Su Jang, *RI-Biomics Research & Development Team, Korea Atomic Energy Research Institute, Jeongeup, Jeonbuk 580-185, Korea*
E-mail: jangbs@kaeri.re.ke

lipid-based nanoparticle, and was successfully used to deliver a gene to the neovasculature for a tumor regression (13). PIA was also modified with carbamate linkers to conjugate to fluorescein isothiocyanate, an optical probe, for the optical imaging of tumors (14). In this study, PIA was labeled with ^{99m}Tc using organometallic ^{99m}Tc(I) aqua ions, $[^{99m}Tc(CO)_3(H_2O)_3]^+$, as a ^{99m}Tc(I) labeling reagent. Recently, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ has been intensively investigated to incorporate a small $[^{99m}Tc(CO)_3]^+$ moiety to biomolecules with various bidentate and tridentate ligands (15-23). To label PIA with $[^{99m}Tc(CO)_3]^+$, an aminodiacetic acid (ADA) moiety was conjugated to PIA by reacting it with tris-succinimidyl aminotriacetate (TSAT). The tridentate aminodiacetic acid moiety of the PIA conjugates was facially coordinated to ^{99m}Tc(I) of $[^{99m}Tc(CO)_3]^+$ by displacing the tris-aqua molecules. The resulting $^{99m}Tc(CO)_3$-ADA-PIA was subjected to a biodistribution in nude mice bearing receptor-positive M-21 human melanoma xenografts.

The aim of this research was to label PIA with ^{99m}Tc and test its receptor-targeting properties in nude mice bearing a receptor-positive tumor.

MATERIALS AND METHODS

Synthesis of PIA-$COCH_2N(CH_2CO_2H)_2$. We optimized the reaction conditions to acylate the amino group of PIA with one of the three activated esters of TSAT and to hydrolyze the remaining two esters to produce an aminodiacetic acid moiety: PIA (25 mM) was reacted with TSAT at a PIA-to-TSAT molar ratio ranging from 1.2 : 1 to 5 : 1 at pH 8.5 for 1 hr at room temperature (Fig. 1).

Preparation of fac-[$^{99m}Tc(CO)_3$-ADA-PIA]. The resulting product PIA-$COCH_2N(CH_2CO_2H)_2$ (PIA-ADA) was reacted with $[^{99m}Tc(CO)_3(H_2O)_3]^{+1}$ at pH 4.5 for 45 min at 70°C to label the tridentate aminodiacetic acid moiety of the products with $[^{99m}Tc(CO)_3]^{+1}$ by displacing the tris-aqua groups of $[^{99m}Tc(CO)_3(H_2O)_3]^{+1}$ (13,14). The labeled product was purified sequentially on Sep-Pak C-18 and Sep-Pak QMA anion exchange cartridges. The radiochemical purity (> 95%) was confirmed by gradient C-18 reverse-phase HPLC (Ace 5 C18 column, 4.6 × 100 mm, 5 mm, MAC-MOD Analytical Inc.). HPLC solvents consisted of a 0.05 M triethylammonium phosphate (TEAP) buffer, pH 2.25 (solvent A), and acetonitrile (solvent B). The applied gradient was 0 to 2 min, 100% A; 2 to 5 min, from 100 to 80% A; 5 to 12 min, from 80 to 65% A; 12 to 20 min, from 35 to 100% B; and 20 to 25 min, 100% B. The HPLC showed one peak with a retention time of 10.5 min for $^{99m}Tc(CO)_3$ADA-PIA (Fig. 2).

Stability study. The stability of $^{99m}Tc(CO)_3$ADA-PIA was tested in a 0.15 M sodium phosphate buffer (PB) at pH 7.2

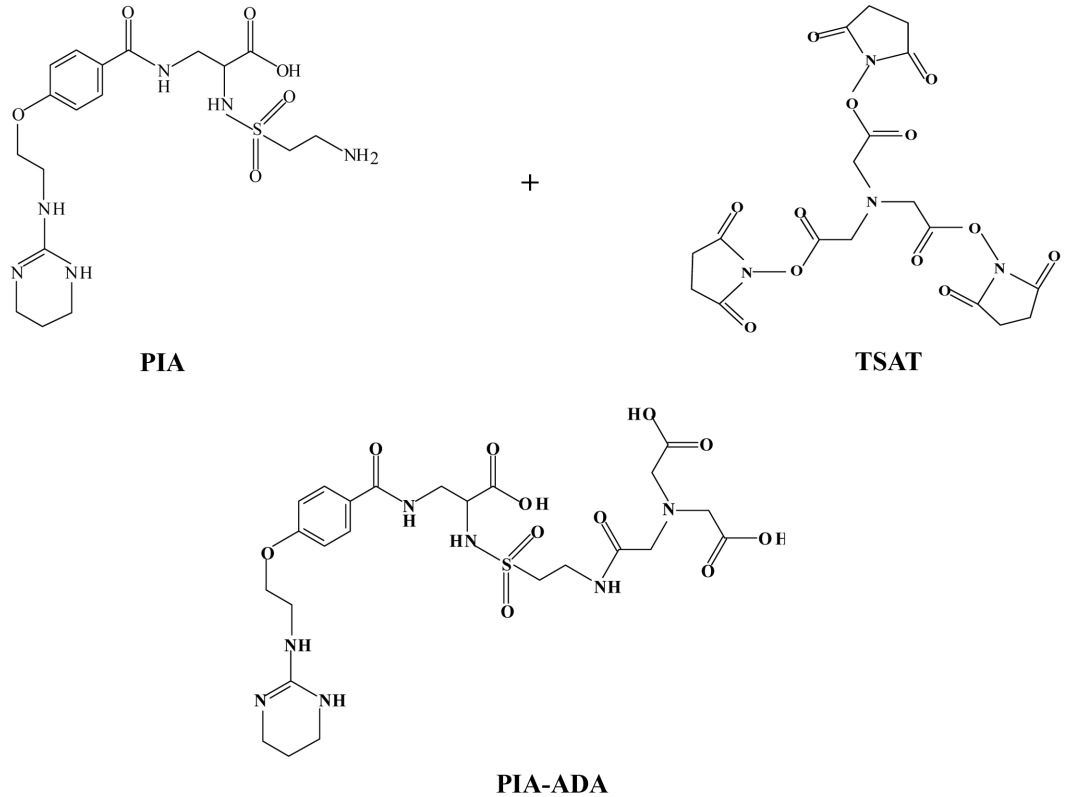

Fig. 1. Conjugation scheme.

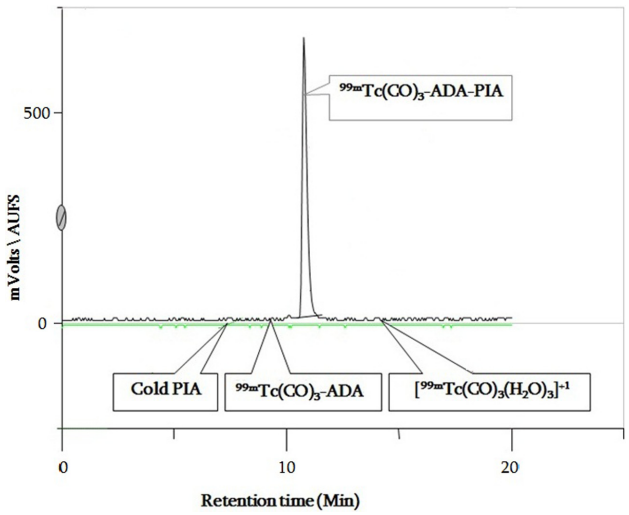

Fig. 2. Reverse-Phase HPLC profile of ^{99m}Tc(CO)$_3$-ADA-PIA on ACE5 C18 column.

and against two more common trans-chelating reagents, histidine (20 mM in PB at pH 7.2) and cysteine (20 mM in PB at pH 7.2) at 200-times molar excess to the ^{99m}Tc label at 37°C. The solutions were analyzed by RP HPLC as described above.

Biodistribution study. Biodistribution was performed in nude mice (n = 5 per time point) bearing receptor-positive M-21 human melanoma xenografts. The mice received intravenous ^{99m}Tc(CO)$_3$-ADA-PIA (5 μCi/< 0.1 μg). The animals were euthanized at 0.33, 1, and 2 hrs after injection for the biodistribution study. In a separate experiment, a group of mice were co-injected with ^{99m}Tc(CO)$_3$-ADA-PIA with 200 μg of PIA and euthanized at 1 hr to test if the tumor uptake was blocked with an excess amount of PIA.

RESULTS

PIA was conjugated with TSAT to generate an aminodiacetic acid moiety as iminodiacetic acid is one of the smallest tridentates that can complex with [^{99m}Tc(CO)$_3$]$^+$. The conjugation reaction of PIA to TSAT at a molar ratio of 1.2 : 1 produced a higher yield of mono PIA conjugated to TSAT as compared to the reaction at a PIA-to-TSAT molar ratio of 5 : 1. This result was also reflected in the radiolabeling yield; the product from a molar ratio of 1.2 : 1 resulted in a 78% labeling yield, whereas the remaining activity was complexed to nitirilotriacetic acid, the hydrolyzed product of TSAT. ^{99m}Tc(CO)$_3$-ADA-PIA was stable in PB for 21 hrs, but lost 7.9, 11.5, and 25.4% of the radioactivity to histidine at 3, 6, and 21 hrs. It did not lose ^{99m}Tc to cysteine but produced two polar degraded products composed of 14.6, 37.5, and 100% of the total at 3, 6, and 21 hrs, respectively. The result indicates that cysteine, a reducing agent, is harmful to this product. Since the labeled product was quite stable for 3 hrs under harsh conditions, a biodistribution study followed. In the tumor bearing mice, ^{99m}Tc(CO)$_3$-ADA-PIA accumulated rapidly in receptor-positive tumors with a peak uptake of 1.2 ± 0.4% ID/g at 20 min, while being cleared rapidly from blood primarily through the hepatobiliary system with 6.5 ± 0.8 and 25.4 ± 2.8% ID/g localized in the liver and intestine, respectively, at that time (Fig. 3). At 20 min, the tumor-to-organ ratios were 1.84, 0.18, 0.69, 1.01, 2.89, 0.22, 0.04, 2.05, and 4.10 for the blood, liver, kidney, lung, heart, stomach, intestine, bone, and muscle, respectively. At 1 hr, the tumor uptake was 0.47 ± 0.15% ID/g,

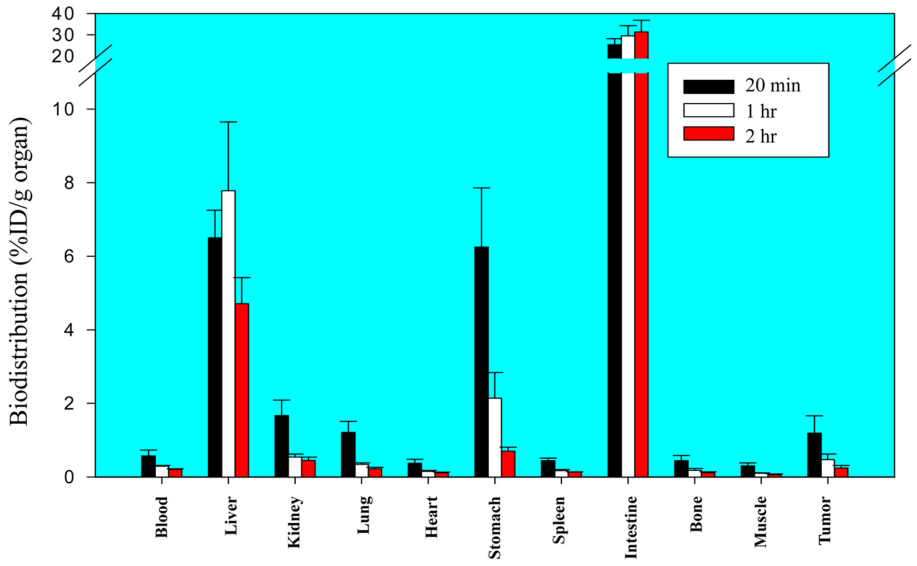

Fig. 3. Biodistribution (% ID/g organ) of ^{99m}Tc(CO)$_3$-ADA-PIA.

but decreased to 0.12 ± 0.02% ID/g when co-injected with an excess amount of PIA.

DISCUSSION

The goal of this research was to synthesize ^{99m}Tc labeled peptidomimetic integrin $\alpha_v\beta_3$ antagonists that would target $\alpha_v\beta_3$ receptor-positive tumors and produce high target-to-nontarget radioactivity ratio at an early time after injection. In this research, the effect of the receptor binding affinity of the ^{99m}Tc labeled peptidomimetic antagonist on the *in vivo* kinetics in the tumor was investigated. TSAT was used as a bifunctional chelator. The conjugation reaction was optimized at a PIA-to-TAST molar ratio of 1.2.

The results of a biodistribution and blocking study with an excess amount of PIA indicated that ^{99m}Tc(CO)$_3$-ADA-PIA was accumulated through a receptor mediated uptake. The tumor-to-organ ratios did not improve much over time because the labeled PIA was not retained well in the tumor, which is perhaps due to its low affinity to the receptor.

In conclusion, a peptidomimetic antagonist to integrin was successfully labeled with ^{99m}Tc. It accumulated in the tumor, mediated through specific receptor binding. However, the uptake in the tumor was very low because of its low blood concentration that is ascibed to its rapid excretion into the hepatobiliary system. This study suggests that to be useful as a tumor detection agent, it is necessary to improve the receptor-binding affinity by synthesizing the oligomers of PIA and increase the hydrophilicity of the product by glycosylation and PEGylation to minimize the rapid uptake into the hepatobiliary system.

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