Published in final edited form as:

Curr Radiopharm. 2010 January 1; 3(1): 1–8. doi:10.2174/1874471011003010001.

^{99m}Tc-labeling of Peptidomimetic Antagonist to Selectively Target $\alpha_v\beta_3$ Receptor-Positive Tumor: Comparison of PDA and EDDA as co-Ligands

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Abstract

Objectives—The aim of this research was to synthesize radiolabeled peptidomimetic integrin $\alpha_v\beta_3$ antagonist with ^{99m}Tc for rapid targeting of integrin $\alpha_v\beta_3$ receptors in tumor to produce a high tumor to background ratio.

Methods—The amino terminus of 4-[2-(3,4,5,6-tetra-hydropyrimidin-2-ylamino)-ethoxy] benzoyl-2-(S)-[N-(3-amino-neopenta-1-carbamyl)]-aminoethylsulfonyl-amino- β -alanine hydrochloride (IAC) was conjugated with N-hydroxysuccinimide ester of HYNIC and labeled with ^{99m}Tc using tricine with either 1,5-pyridinedicarboxylic acid (PDA) or ethylenediamine-N,N'-diacetic acid (EDDA) as the co-ligand. The products, ^{99m}Tc EDDA₂/HYNIC-IAC (P1) and ^{99m}Tc PDA (tricin)/HYNIC-IAC (P2) were subjected to *in vitro* serum stability, receptor-binding, biodistribution and imaging studies.

Results—P1 and P2 were synthesized with an overall yield of >80%. P1 was slightly more stable than P2 when incubated in serum at 37 °C for 18 hrs (84 vs 77% intact). The *In vitro* receptor-binding of P1 was higher than that of P2 (78.02 ± 13.48 vs 51.05 ± 14.05%) when incubated with $\alpha_v\beta_3$ at a molar excess (0.8 μ M). This receptor binding was completely blocked by a molar excess of an unlabeled peptidomimetic antagonist. Their differences shown in serum stability and the receptor-binding appeared to be related to their biological behaviors in tumor uptake and retention; the 1 h tumor uptakes of P1 and P2 were 3.17±0.52 and 2.13±0.17 % ID/g, respectively. P1 was retained in the tumor longer than P2. P1 was excreted primarily through the renal system whereas P2 complex was excreted equally *via* both renal and hepatobiliary systems. Thus, P1 was retained in the whole-body with 27.25 ± 3.67% ID at 4 h whereas 54.04 ± 3.57% ID of P2 remained in the whole-body at 4 h. This higher whole-body retention of P2 appeared to be resulted from a higher amount of radioactivity retained in liver and intestine. These findings were supported by imaging studies showing higher tumor-to-abdominal contrast for P1 than for P2 at 3 h postinjection.

Conclusions—P1 showed good tumor targeting properties with a rapid tumor uptake, prolonged tumor retention and fast whole-body clearance kinetics. These findings warrant further investigation

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of the HYNIC method of ^{99m}Tc labeling of other peptidomimetic antagonists using EDDA as a coligand.

Keywords

HYNIC method of ^{99m}Tc -labeling; peptidomimetic $\alpha_v\beta_3$ antagonist; EDDA and PDA as co-ligand; Tumor Imaging

INTRODUCTION

Integrin $\alpha_v\beta_3$ is a heterodimeric transmembrane glycoprotein and a receptor for extracellular matrix proteins including vitronectin, fibronectin and fibrinogen which contain an arginine-glycine-aspartic acid (RGD) sequence [1,2]. Integrin $\alpha_v\beta_3$ is overexpressed in tumor induced angiogenic vessels and various malignant human tumors. To assess angiogenesis and receptor status in tumors by selective targeting of this receptor, various RGD peptides and peptidomimetic antagonists have been labeled with gamma and positron emitters for scintigraphic detection [2–9] and beta emitters for radiotherapy of tumors [10]. Steady progress has been reported in optimizing the labeling methodologies. To increase tumor-to-non-tumor tissue ratios, especially for the tumor-to-liver ratio, the organ clearance pharmacokinetics was optimized by increasing the hydrophilicity of the product *via* glycosylation and PEGylation [11–14]. Dimers and tetramers of cyclic RGD derivatives have been synthesized to increase the receptor binding affinity, thereby improving the tumor targeting and retention [3,15,16].

We have investigated the use of peptidomimetic antagonists, 4-[2-(3,4,5,6-tetrahydropyrimidine-2-ylamino)ethyloxy]benzoyl-2-(S)-aminoethylsulfonyl-amino- β -alanine (IA) and 4-[2-(3,4,5,6-tetrahydropyrimidin-2-ylamino)-ethyloxy]benzoyl-2-(S)-[N-(3-aminoneopenta-1-carbamyl)]-aminoethylsulfonylamino- β -alanine hydrochloride (IAC), which is a higher affinity carbamate derivative of a first generation peptidomimetic integrin $\alpha_v\beta_3$ antagonist, IA, for molecular imaging of tumors *via* binding to $\alpha_v\beta_3$ receptor. We previously labeled IA and IAC with ^{111}In using 2-(*p*-isothiocyanatobenzyl)-DOTA as a bifunctional chelator and investigated comparative *in vivo* studies in nude mice bearing the receptor-positive M21 tumors [17]. The comparative biodistribution and imaging studies indicated that both ^{111}In labeled-IA and -IAC using DOTA as a bifunctional chelator were primarily excreted *via* the renal system and a minor portion of the labeled injectants was excreted *via* the hepatobiliary route. The biodistribution study also showed that the ^{111}In labeled-IAC with a higher affinity produced higher tumor uptake and higher tumor-to-non-tumor radioactivity ratios than the ^{111}In labeled-IA. The tumor was clearly visualized by static imaging 3 hrs after the injection of the ^{111}In labeled-IAC. In this current research, we investigated the ^{99m}Tc labeling of IAC using the HYNIC method with either EDDA or PDA as a co-ligand and studied the biodistribution of the resulting products in nude mice bearing the receptor-positive M21 tumors. We selected the HYNIC method because this method was shown to produce a hydrophilic, stable ^{99m}Tc labeled RGD with a high affinity to $\alpha_v\beta_3$ receptor *in vitro* and good tumor targeting properties *in vivo* [18–24].

MATERIALS AND METHODS

Synthesis of IAC-HYNIC

The synthesis of 4-[2-(3, 4, 5, 6-tetrahydropyrimidin-2-ylamino)-ethyloxy]benzoyl-2-(S)-[N-(3-amino-neopenta-1-carbamyl)]-aminoethylsulfonylamino- β -alanine hydrochloride (IAC) was reported previously [25]. HYNIC-NHS (1.67 mM; Solulink, San Diego, CA) was conjugated to the amino terminus of IAC (16.7 mM), by reacting the two reagents with gentle shaking in a solvent mixture containing 67% DMF, 17% DMSO and 16% aqueous medium with the final concentration of 83 mM sodium bicarbonate overnight at room temperature (Fig.

1). The reaction resulted in the IAC-HYNIC conjugate with a 97% yield calculated based on HYNIC-NHS. The yield of IAC-HYNIC was determined based on the distribution of ^{99m}Tc between IAC-HYNIC and HYNIC as described below.

^{99m}Tc labeling of IAC-HYNIC using EDDA or PDA as a co-ligand

In a rubber-sealed vial, IAC-HYNIC (33.4 μM) was incubated with $^{99m}\text{TcO}_4^{-1}$ (31 mCi; 1147 MBq) in 0.3 ml aqueous medium at pH 6 containing tricine (112 mM, 20 mg/ml), sodium succinate (25 mM) and stannous chloride dehydrate (100 $\mu\text{g/ml}$) at 37 °C for 30 min. To this solution, 0.3 ml aqueous medium containing 6 mg of EDDA or PDA was added. Then the mixture was heated at 80°C for 25 min (Fig. 1). As reported for the ^{99m}Tc complex of HYNIC-RGD peptides [20,24,26,27], we hypothesize that the ^{99m}Tc labeling of HYNIC-IAC in the presence of EDDA produced a ^{99m}Tc complex involving one or two EDDA molecules bonded to ^{99m}Tc -HYNIC-IAC core (P1) and the ^{99m}Tc labeling in the presence of PDA produced a ternary ^{99m}Tc complex involving each of PDA and Tricine bonded to ^{99m}Tc -HYNIC-IAC core (P2) (Fig. 1).

The labeling yield was determined by reverse-phase HPLC (Ace 5 C18 column, 4.6×100 mm, 5 μm , MAC-MOD Analytical Inc., Chadds Ford, PA). HPLC solvents consisted of 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; 20 to 25 min, 100% B. The labeled products were purified on a Sep-Pak C-18 cartridge. Each of the labeled products was diluted to 20 ml with distilled water and was loaded on a Sep-Pak C-18 cartridge (Long Body Sep-Pak plus, Waters, Milford MA); the Sep-Pak C-18 cartridge was preconditioned prior to the sample purification by washing with 20 ml of acetonitrile followed by 20 ml of distilled water. The cartridge was eluted stepwise with distilled water (10 ml), 5% acetonitrile in water (5 ml) and 10% acetonitrile in water (5 ml) and 20% acetonitrile in water. The product eluted out with 20% acetonitrile was collected. The organic solvent was removed by lyophilization and the product was re-dissolved in 0.02 M sodium phosphate in 0.15 M sodium chloride, pH 7.2 (PBS).

Stability study

The stability was tested in serum; 400 μl of mouse serum with 0.04% sodium azide was mixed with 50 μl of 0.5 M sodium phosphate, pH 7.2 and 50 μl (116 μCi , 4.29 MBq) of P1 or P2. The solution was incubated at 37°C. Aliquots were taken out at designated time points and analyzed by size exclusion HPLC equipped with a TSK gel G3000SW_{XL} column (7.8×300 mm, 5 μm , TOSOH Bioscience, Japan; 0.067 M sodium phosphate/0.10 M potassium chloride, pH 6.8; 1.0 ml/min), a UV monitor and an online flow radioactivity detector (Bioscan Inc., Washington DC).

Receptor-binding assay

To assess the receptor-binding ability, the ^{99m}Tc labeled products (1.1×10^6 cpm, <2.5 pmol) were incubated with various amounts (2.6 to 51.2 pmol) of human integrin $\alpha_v\beta_3$ (MW 237,000; Chemicon, Temecula, CA) in a total volume of 64 μl PBS, pH 7.2 at 37 °C for 3 hrs [9]. To assess the percentage of non-specific binding, the binding assay was performed in the presence of a 250 times molar excess of IA to integrin $\alpha_v\beta_3$. The binding percentage to the integrin $\alpha_v\beta_3$ was analyzed by size exclusion HPLC equipped with a TSK gel G3000SW_{XL} column. The retention time of the receptor-bound and the free ^{99m}Tc labeled antagonists was 7 min and 15 min, respectively.

Tumor Model

The human M21 melanoma cell line expressing $\alpha_v\beta_3$ was used to make an *in vivo* tumor model. Human M21 melanoma cells initially received from Dr. David Cheresch (Scripps Research Institutes, La Jolla, CA) were cultured in RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin under a humidified atmosphere with 5% CO₂ [9]. Tumor xenografts were established by *s.c.* inoculation of 3×10^6 cells into the right flank of athymic mice (NCI-DCT, Frederick, MD).

Biodistribution Study

Animal experiments were performed under an NIH Animal Care and Use Committee approved protocol. Biodistribution studies were performed in nude mice (n=4~15 per time point) bearing receptor-positive M21 human melanoma xenografts. The mice received intravenous (*i.v.*) P1 or P2 (6.25 μ Ci, 0.231 MBq/6 pmol in 0.2 ml PBS containing 1% BSA when the tumor size was approximately 200 mm³. The mice were euthanized at designated times after injection for the biodistribution study. In a separate experiment, a group of mice was coinjected with 0 ~ 200 μ g of unlabeled IA and euthanized at 1 h to test if the tumor uptake was blocked by the excess amount of IA.

Imaging

Nuclear imaging study of tumor-bearing mice was performed using a micro-SPECT single photon emission computed tomography system (A-SPECT, Gamma Medica Instruments, Northridge, CA) with a 2 mm pin-hole collimator for high resolution animal imaging. The camera was equipped with an array of 2 mm X 2 mm X 6 mm NaI (TI) pixels coupled to a position-sensitive photo-multiplier tube readout. Mice were *i.v.* injected with 33.3 MBq (900 μ Ci) of either P1 or P2. Then the animals were anaesthetized with ketamine (60 mg/kg)/xylazine (10 mg/kg) immediately before being placed supine in a gantry positioned in front of the pin-hole collimator, and they were centered on the field of view of the micro-SPECT. The static imaging was performed for 10 ~ 40 min at 3 h post-injection.

Statistical analysis

Statistical analysis was performed using ANOVA [9] for comparing multiple groups, and Student's *t* test was performed for unpaired data between two groups. All tests were two-sided, and a probability value (*p*) of less than 0.05 was considered significant.

RESULTS

In vitro studies

The IAC-HYNIC was synthesized with a 97% yield based on HYNIC-NHS and was labeled with ^{99m}Tc using EDDA or PDA as a co-ligand without further purification. The conjugation yield of HYNIC to the amino terminus of IAC was proportional to the concentration of IAC as determined by the distribution of ^{99m}Tc between HYNIC-IAC and HYNIC. A quantitative conjugation yield was obtained when the IAC concentration was > 20 mM and the reaction was performed at an IAC to chelator molar ratio of 10 at pH 8.4 overnight. This high conjugation yield rendered the purification step of HYNIC-IAC from the free chelator unnecessary. The ^{99m}Tc labeled product P1 or P2 was purified on a Sep-Pak C-18 cartridge and were eluted out with 20% acetonitrile. The organic solvent was then removed by lyophilization and the product was redissolved in 0.02 M sodium phosphate-0.15 M NaCl (PBS). The radiochemical purity of the purified P1 or P2 was > 97% (Fig. 2). The radiolabeled products were stable in PBS with ^{99m}Tc radioactivity associated with P1 and P2 for an 18-h period of incubation. The stability study in serum indicated that at 18 h, 13% and 14% of total ^{99m}Tc radioactivity were associated with a protein with its HPLC retention time identical

to albumin and 3% and 9% of ^{99m}Tc radioactivity were associated with $^{99m}\text{TcO}_4^{-1}$ for P1 and P2, respectively (Fig. 3). The receptor-bindability analyzed by size exclusion HPLC showed that $78.02 \pm 13.48\%$ ($n = 4$) of P1 and $51.05 \pm 14.05\%$ ($n = 4$) of P2 bound to $0.8 \mu\text{M}$ $\alpha_v\beta_3$ *in vitro*. The receptor-binding was completely blocked by a molar excess of unlabeled $\alpha_v\beta_3$ antagonist.

Animal studies

Both P1 and P2 accumulated rapidly in the receptor-positive tumor with the uptake values of 3.17 ± 0.52 and $2.13 \pm 0.17\%$ ID/g at 1 h, respectively. The tumor uptake of P1 at 1 h was blocked by unlabeled IA in a concentration dependent manner (Fig. 4). The co-injection of 200 μg of the antagonist decreased the tumor uptake values of P1 and P2 to $0.68 \pm 0.06\%$ ID/g and $0.96 \pm 0.30\%$ ID/g at 1 h, respectively ($p < 0.004$). This indicated that the tumor uptake was receptor mediated (Fig. 5). There were differences shown in their washout rates from the tumor; the tumor uptake value of P1 remained relatively unchanged for a 4-h period whereas the radioactivity of P2 in the tumor was washed out with 62% of P2 at 1 h remained in the tumor at 4 h ($p < 0.00005$) (Fig. 5). Differences were also found in the whole-body retention; P1 was retained in the whole-body with $39.58 \pm 2.91\%$ ID at 1 h and $27.25 \pm 3.67\%$ at 4 h, indicating that it was excreted primarily *via* the renal system. On the other hand, P2 remained in the whole-body with $57.84 \pm 5.15\%$ ID at 1 h and $54.04 \pm 3.57\%$ ID at 4 h, indicating that it was excreted to a larger extent *via* the hepatobiliary system (Fig. 6). Comparative biodistribution studies also showed significantly higher accumulation of P2 in liver and intestine than P1 for a 4-h period. The tumor-to-nontumor ratios of both P1 and P2 increased over time, indicating preferential retention of radioactivity in tumor over most other organs except the intestine. Comparing the tumor-to-nontumor ratios of the two labels, the increases in the ratios of P1 were larger than those of P2 (Table 1). These biodistribution results were further substantiated by imaging studies (Fig. 7). The static anterior imaging performed at 3 h post-injection showed that the tumor areas were much brighter than the surrounding background. The non-tumor associated radioactivity was primarily seen in abdomen and bladder areas for P1. Compared to P1, a much higher background was observed in the P2 images.

DISCUSSION

The goal of this research was to synthesize ^{99m}Tc labeled peptidomimetic antagonists that achieve a high target to non-target radioactivity ratio within a short time after injection. In this study, a second generation peptidomimetic $\alpha_v\beta_3$ antagonist, IAC (IC_{50} 2.94 nM), was conjugated with HYNIC for ^{99m}Tc labeling. We optimized the conjugation reaction at pH 8.4 and an IAC to succinimidyl HYNIC molar ratio of 10 to conjugate HYNIC solely to the amino terminus of IAC and not to the guanidino group of IAC. We believe that under this optimal condition, the guanidino group ($\text{pK}_a \sim 12$) is completely protonated and thus, non-reactive with succinimidyl HYNIC. The resulting HYNIC-IAC was labeled with ^{99m}Tc by stepwise reactions to produce either P1 or P2. P1 or P2 was produced when Tricine was first reacted and then EDDA or PDA was added as a coligand. Both P1 and P2 had an overall yield of $>80\%$ yield. Both P1 and P2 were quite stable. P1 was also produced in one step reaction without Tricine. However, the labeling yield from the one step reaction was much lower than the 2-step reaction with Tricine (unpublished observation), similar to that reported by Wang, *et al.* [26]. When P1 and P2 were incubated in serum for 18 h at 37°C , about 13% of the total ^{99m}Tc radioactivity was bound to a serum protein with a HPLC retention time similar to that of albumin, and 4 to 9% of the radioactivity was associated with a peak with a HPLC retention time identical to that of $^{99m}\text{TcO}_4^{-1}$. These results are similar to those reported for the corresponding ^{99m}Tc labeled peptides [28]. The structure of the ^{99m}Tc complex of EDDA/HYNIC has not been unequivocally defined. von Guggenberg, *et al.*, postulated a ^{99m}Tc complex involving two EDDA molecules bonded to ^{99m}Tc -HYNIC core based on the analysis

by Mass Spectroscopy [27] whereas Wang, et al., proposed a ^{99m}Tc complex involving one EDDA molecules bonded to ^{99m}Tc -HYNIC core [26]. A ternary complex of PDA (Tricine)/HYNIC was first postulated by Liu, et al. [20]. However, we have not further investigated on the precise structure of these ^{99m}Tc complexes in this current study.

The *in vivo* studies, P1 showed better tumor targeting properties than P2. Although two ^{99m}Tc radioligands rapidly targeted tumor *via* a receptor-mediated uptake, P1 remained longer in the tumor. It was excreted primarily *via* the renal system, thereby resulting in lower whole-body background radioactivity for a 4-h period. On the other hand, P2 was excreted equally *via* the hepatobiliary and the renal systems, thereby showing higher liver and intestinal radioactivity during a 4-h study. The imaging studies with these two ^{99m}Tc radioligands also clearly visualized tumors at 3 h postinjection. However, a better tumor-to-abdominal contrast was shown with P1. The whole-body clearance of P1 was similar to that of ^{111}In labeled IAC using 2-*p*-isothiocyanatobenzyl-DOTA as a bifunctional chelator [28]. Although the tumor uptake values were lower for P1 than for the ^{111}In labeled IAC, the tumor-to-organ ratios of P1 at 4 h became similar to the corresponding ratios of the ^{111}In labeled IAC at 2 h because of the preferential retention of P1 in the tumor than in the organs. The tumor imaging property of P1 was comparable to that of ^{111}In labeled IAC using 2-*p*-isothiocyanatobenzyl-DOTA as a bifunctional chelator at 3 h postinjection.

CONCLUSION

P1 showed good tumor targeting properties with a rapid tumor uptake kinetics, prolonged tumor retention and rapid whole-body clearance kinetics. These favorable biological behavior in combining with excellent physical properties ($t_{1/2}$ of 6 h and 140 KeV photon energy) of ^{99m}Tc warrant further investigation of ^{99m}Tc labeling for the receptor-mediated tumor targeting utilizing the HYNIC method for other peptidomimetic antagonists with even higher affinity produced by the dimerization or tetramerization of this peptidomimetic antagonist, similar to that shown for cyclic RGDfK derivatives [3,26].

Acknowledgments

This research was supported by the intramural research program of Clinical Center, NIH.

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FIGURE 1. Synthesis of ^{99m}Tc labeled IAC

HYNIC-NHS (1.67 mM) was conjugated to the amino terminus of IAC (16.7 mM) in 83 mM sodium bicarbonate (pH 8.4) at room temperature overnight. IAC-HYNIC was incubated with $^{99m}\text{TcO}_4^{-1}$ in 0.3 ml at pH 6 containing tricine (112 mM, 20 mg/ml), sodium succinate (25 mM) and stannous chloride dehydrate (100 $\mu\text{g}/\text{ml}$) at 37 $^{\circ}\text{C}$ for 30 min. To this solution, 0.3 ml aqueous medium containing 6 mg of EDDA or PDA was added. Then the mixture was heated at 80 $^{\circ}\text{C}$ for 25 min.

FIGURE 2. Reverse-phase HPLC profile of P1 (A) and P2 (B) after Sep-Pak C-18 purification. See the Methods section for the HPLC elution condition.

FIGURE 3.

Stability of P1 and P2 in mouse serum at 37 °C. The formation of ^{99m}Tc -bound serum protein and $^{99m}\text{TcO}_4^{-1}$ increased over time whereas the percentage of P1 (diamond) and P2 (square) decreased. Mean values were plotted over time. Bar, S.D.; $N \geq 3$.

FIGURE 4.

Effect of IA co-injection on tumor uptake (%ID/g) of P1. A group of mice was coinjected with 0, 10 and 200 µg of unlabeled IA and the tumor uptake was determined at 1 h postinjection. Columns, mean; bar, S.D. N = 5.

FIGURE 5. Biodistribution (%ID/g, $n \geq 5$) of P2 (A) and P1 (B) in melanoma M21-bearing nude mice. Columns, mean; bar, SD.

FIGURE 6. Whole body retention (%ID/g, n = 5) of P1 (square) and P2 (circle) in melanoma M21-bearing nude mice at 1, 2 and 4 hour post injection. Mean values were plotted over time. Bar, S.D.

FIGURE 7.

Images of melanoma M21-bearing nude mice at 3 h postinjection. The mice received 33.3 MBq of P1 or P2 by *i.v.* injection. (A) and (B), P1; (C) and (D), P2. The animals were anaesthetized with ketamine (60 mg/kg)/xylazine (10 mg/kg) immediately before imaging. The static image was acquired for 33 min/40 K counts (A), 43 min/40 K counts (B), 17 min/40 K counts (C), and 13 min/40 K counts (D) to visually compare the tumor-to-background ratios.

Table 1

Tumor-to-organ ratios* of P1 and P2 in M21 melanoma bearing nude mice

Organ	Time							
	0.3 h		1 h		2 h		4 h	
	P1 ^a	P1	P2 ^b	P1	P2	P1	P2	P1
Blood	0.90±0.20	1.38±0.48	1.83±0.16	2.19±0.18	2.58±0.25	17.11±5.18	3.15±0.25	
Spleen	0.71±0.07	1.36±0.38	1.09±0.15	1.30±0.12	1.95±0.27	2.83±0.81	1.54±0.40	
Liver	0.72±0.12	0.89±0.27	0.46±0.27	1.02±0.12	0.45±0.30	1.59±0.72	0.40±0.17	
Kidney	0.51±0.12	0.75±0.28	0.62±0.05	0.85±0.08	0.81±0.07	1.22±0.32	0.78±0.04	
Lung	0.72±0.10	1.19±0.43	0.83±0.22	1.50±0.09	1.34±0.51	2.47±0.49	2.71±0.95	
Heart	1.91±0.43	2.48±0.75	2.64±0.36	3.23±0.48	3.85±0.57	7.62±1.99	3.88±0.90	
Stomach	0.54±0.13	0.40±0.20	0.40±0.12	0.43±0.11	0.29±0.09	1.53±0.80	0.27±0.05	
Intestine	0.66±0.07	0.60±0.19	0.15±0.01	0.57±0.07	0.11±0.02	0.46±0.15	0.07±0.00	
Bone	1.29±0.23	2.12±1.17	1.48±0.37	2.46±0.19	2.73±0.54	4.78±1.07	3.76±1.36	
Muscle	3.04±0.68	6.11±1.80	3.85±1.64	4.68±0.31	5.75±1.56	7.84±1.78	6.33±1.57	

^a99mTc EDDA2/HYNIC-IAC (P1)

^b99mTc PDA (tricin)/HYNIC-IAC (P2)

* Ratios were calculated by dividing tumor uptake (% ID/g) by organ uptake (% ID/g)