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Labeling, Stability and Biodistribution Studies of ^{99m}Tc -cyclized Tyr³-octreotate Derivatives

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Abstract

Introduction—To probe the interplay between radiotracer stability and somatostatin receptor affinity, Tyr³-octreotate and six variations of its peptide sequence, for which the Re-cyclized products were previously reported, were radiolabeled with ^{99m}Tc and investigated for their in vitro stability.

Methods—Radiolabeling of the peptides was effected by ligand exchange from ^{99m}Tc -glucoheptonate, and the desired products were purified by radio-RP-HPLC. The in vitro stability in phosphate-buffered saline, mouse serum, and cysteine solutions at physiological temperature and pH for all seven ^{99m}Tc -cyclized peptides was determined by radio-RP-HPLC and radio-TLC. Normal CF-1 mouse biodistribution studies were performed for three of the ^{99m}Tc -cyclized peptides.

Results—Based on the fully characterized Re-cyclized peptide analogues, four ^{99m}Tc -coordination motifs were proposed for the ^{99m}Tc -cyclized peptides. Technetium- ^{99m}Tc -cyclized Tyr³-octreotate derivatives with N₂S₂ metal coordination modes and large metal ring sizes were susceptible to oxidation and loss of ^{99m}Tc in the form of $^{99m}\text{TcO}_4^-$, as evidenced by their instability in the various solutions under physiological conditions (15–58% intact at 24 h). As anticipated, the addition of a third cysteine to the sequence stabilized the ^{99m}Tc metal coordination, and peptides with NS₃ coordination modes remained >85% intact out to 24 h. No significant differences were observed in the biodistribution studies performed with three peptides of varying stabilities.

Conclusions—Improvements in stability were not sufficient to outweigh the low somatostatin receptor affinity for the peptides in this study. Further improvements in the peptide sequence and/or metal coordination are needed to result in a radiodiagnostic/radiotherapeutic pair for targeting the somatostatin receptor.

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Keywords

Tc-99m; Tyr³-octreotate; somatostatin receptor; peptide

1. Introduction

Technetium-99m (^{99m}Tc) and rhenium-186/188 (^{186/188}Re) have suitable nuclear properties for imaging and therapy, which make them an attractive pair of radionuclides in radiodiagnostics and radiotherapeutics, respectively. Although the use of bifunctional chelators is far and away the most common approach to incorporating these radionuclides into somatostatin receptor (SSTR)-targeting peptides,[1–7] other approaches have also been investigated. For example, Fridkin et al. used a hemi-chelator approach where bidentate chelators, positioned on the peptide backbone at the *N*- and *C*-termini of the somatostatin peptide analogues studied, effected simultaneous metal/radiometal labeling and peptide cyclization.[8] We and others have reported the direct incorporation of the radiometal into the disulfide bridge of octreotide and its derivatives.[9–12]

We recently synthesized, characterized and measured the somatostatin subtype 2 receptor (SSTR2) affinity of 11 new Re-cyclized peptides, nine of which were based on the Tyr³-octreotate sequence (TATE; D-Phe-c(Cys-**Tyr-D-Trp-Lys-Thr**-Cys)-Thr(OH); SSTR binding sequence in bold).[13] The modifications made to the TATE sequence were designed to tighten metal coordination and distance the metal center from the SSTR pharmacophore, in an effort to increase the affinity for SSTR2 (from an IC₅₀ of 29 nM for Re-TATE, [14]) and to improve the stability of the peptide metal center (in vitro and in vivo instability were observed at the radiotracer level for ^{99m}Tc-TATE, [9]). Though the modifications resulted in a number of Re metal center coordination modes, none of the other Re-cyclized TATE derivatives demonstrated SSTR2 affinities in the nanomolar range.

In this paper, the results of in vitro stability studies of six TATE-based peptide sequences radiolabeled with ^{99m}Tc, which are analogous to six of the Re-cyclized peptides we previously reported,[13,14] will be discussed. The studies presented herein explore the effects of TATE sequence modifications on ^{99m}Tc radiolabeling and in vitro stability, and thereby complement our macroscopic, nonradioactive Re-cyclized peptide studies. The modifications in this series include acetylation of the peptide *N*-terminus, Cys replacement of DPhe¹, insertion of a third Cys into the sequence, and use of a synthetic bidentate Cys residue. A subset of three ^{99m}Tc-TATE peptides was further investigated in mouse biodistribution studies to probe the interplay between radiotracer stability and SSTR affinity.

2. Materials and Methods

2.1. General

Reagents and solvents were purchased from VWR Scientific Products (St. Louis, MO), Fluka (Milwaukee, WI), Fischer Scientific (Pittsburgh, PA), Sigma-Aldrich (St. Louis, MO), and ACROS-Organics (Geel, Belgium). All reagents and solvents were HPLC grade or of the highest purity obtainable and were used without further purification. Radiotracer reactions were monitored and purified on a Waters 626 chromatograph reversed-phase HPLC (RP-HPLC) equipped with a Canberra NaI(Tl) well detector (Meriden, CT) for radioactivity detection and Waters 2487 Dual λ Absorbance Detector for UV detection at 214 and 280 nm. Radio-RP-HPLC was performed on a Grace Vydac (Hesperia, CA) Protein & Peptide C₁₈ column (300 Å, 0.46 × 25 cm, 5 μ m) attached to a Grace Vydac High Performance Guard Column. Linear gradients of solvent B in solvent A (A: 0.1% TFA in water; B: 0.1% TFA in acetonitrile) with a 1 mL/min flow rate were used. Thin layer

chromatography (TLC) was conducted with Whatman MKC18F silica gel plates (60 Å, 2.5 × 7.5 cm, 200 µm thickness) obtained from EM Science (Gibbstown, NJ) and C₁₈ silica gel plates (aluminum backed, 5 × 10 cm, 150 µm thickness) obtained from Sorbent Technologies (Atlanta, GA). TLC plates were developed with either 10% ammonium acetate (w/v) or 1:1 solvent A:solvent B, and radio-TLC detection was accomplished using a Bioscan (Washington, DC) AR-2000 TLC Imaging Scanner.

2.2. Synthesis and characterization of ^{99m}Tc-peptides

TATE and six derivatives of the TATE peptide sequence (compounds **1–6**, Table 1) were prepared in the reduced (linear) form by standard solid-phase 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.[13,14] The linear peptides were purified by semi-preparative RP-HPLC using a multi-step gradient to separate the desired products from the impurities present in the crude sample. The collected fractions, shown to be pure by LC-ESI-MS characterization, were combined, lyophilized and stored at –20 °C.

The purified linear peptides were cyclized with ^{99m}Tc by ligand exchange from ^{99m}Tc-glucosheptonate, as previously published.[9] Briefly, 600 µL of degassed 0.2 M sodium glucosheptonate containing 0.4 mg stannous chloride was added to 200 µL of ^{99m}TcO₄[–] (~10–30 mCi, obtained from Mid-America Isotopes Inc., Ashland, MO). After a few minutes at room temperature, 10 µL of a 1 mg/mL solution of reduced analogue in water was added. The reaction was heated at 60 °C for 30 min following pH adjustment to 7.9–8.1 using 0.1 M NaOH. The formation of the desired ^{99m}Tc-cyclized peptides was confirmed by RP-HPLC co-injection and co-elution of the isolated ^{99m}Tc-products with their non-radioactive Re-cyclized analogues,[13,14] based on retention times using two detectors (UV and NaI(Tl)). The collected purified fractions were combined with 1–2 mg of gentisic acid, concentrated by centrifugal evaporation for 30 min to ensure removal of the organic solvent components, and diluted in a solvent appropriate for either in vitro stability or in vivo biodistribution studies.

2.3. In vitro stability studies

Each of the seven ^{99m}Tc-cyclized peptides was tested for in vitro stability in various solutions at physiological conditions (pH 7.4 and 37 °C). The purified and concentrated fractions of the radiotracers were diluted to 1 mL by adding phosphate buffered saline (PBS), clarified mouse serum (Sigma, St. Louis, MO), 1 mM cysteine, or 10 mM cysteine solution. Following pH adjustment to 7.4 (using 1 M and 0.1 M NaOH), the diluted solutions were incubated at 37 °C. Aliquots of the incubation mixtures were removed at various time points (1, 2, 4 and 24 h) and analyzed by RP-HPLC to monitor stability (percent of intact radiolabeled peptide remaining). Replicate measurements were obtained by radio-TLC with C₁₈ plates using two developing solutions: 10% ammonium acetate (w/v), to separate intact peptide at the origin (along with colloids, ^{99m}TcO₂) from ^{99m}TcO₄[–] near the solvent front, and 1:1 solvent A: solvent B, to separate intact peptide near the solvent front (along with ^{99m}TcO₄[–]) from colloids at the origin.

2.4. Biodistribution studies

All animal studies were conducted in compliance with a protocol approved by the Animal Care and Use Committee of the University of Missouri-Columbia Animal Care Quality Assurance Office. Two of the ^{99m}Tc-cyclized peptides, ^{99m}Tc-**1** and ^{99m}Tc-**2**, were selected for in vivo experiments. Dilution of the concentrated HPLC-purified radiotracers with 0.1% Tween 80 (v/v) in normal saline, followed by pH adjustment to ~5 using 1 M NaOH, resulted in the desired radiotracer concentration with a suitable pH for use in animals. Biodistribution studies were performed in 6 week old normal female CF-1 mice following tail vein injection of ~10–12 µCi of radiotracer. Mice were euthanized by cervical

dislocation at 1, 4 and 24 h post injection, and the organs and tissues were removed and counted on a Wallac 1480 Wizard 3" automated gamma counter (PerkinElmer Life Sciences, Gaithersburg, MD). A 1 h blocking experiment was also performed by co-injecting the radiotracer with 150 µg of disulfide-cyclized TATE.

3. Results and Discussion

3.1. Synthesis and characterization of ^{99m}Tc -peptides

Seven linear peptides (Table 1) were radiolabeled through metal cyclization with ^{99m}Tc and monitored for in vitro stability. Compounds **1–6** are derivatives of the TATE sequence, and we recently described their Re-cyclized analogues.[13,14] The synthesis and use of ^{99m}Tc -TATE was previously reported,[9] however it was repeated herein for direct comparison under the same in vitro conditions as the ^{99m}Tc -cyclized products of TATE derivatives **1** through **6**.

The generator-produced $^{99m}\text{TcO}_4^-$ was reduced to oxidation state +5 using SnCl_2 and stabilized by temporary chelation to glucoheptonate. Linear compounds **1–6** were then radiolabeled and cyclized with $^{99m}\text{TcO}_3^{3+}$ via transchelation from ^{99m}Tc -glucoheptonate. The progress of the ^{99m}Tc -cyclization reactions was monitored by RP-HPLC using a NaI(Tl) detector. Co-injections of the crude reaction mixtures with the non-radioactive Re-cyclized standards, previously characterized by LC-ESI-MS and 2-D NMR [13,14] and monitored by UV at 214 and 280 nm, allowed the identification of the desired ^{99m}Tc products. The peaks of interest were collected with greater than 98% radiochemical purity, and gentisic acid was added to prevent radiolysis. The ^{99m}Tc -cyclization reactions were reproducible and, although low yielding (~15–25%), resulted in sufficient amounts of the desired ^{99m}Tc -cyclized peptides for use in stability and animal studies.

Each of the ^{99m}Tc -labeled TATE derivatives co-eluted by HPLC with the analogous Recyclized standard, suggesting that the ^{99m}Tc -peptides investigated in this study are similar in structure to their Re-cyclized counterparts. We believe that the ^{99m}Tc -labeled TATE derivatives have the same metal coordination motifs as the Re-cyclized analogues; however, their structures were not characterized by more rigorous techniques such as ^{99g}Tc NMR. The Re-cyclized complexes of TATE, **1** and **6** were reported to have different versions of an N_2S_2 metal coordination system.[13,14] The metal in the TATE and derivative **1** complexes was coordinated to the two Cys thiols (Cys^2 and Cys^7) and the amide nitrogen of Tyr^3 , with the final coordination site being filled by the *N*-terminal amine for TATE and the Thr^6 amide nitrogen for **1**. [14] For derivative **6**, the single Cys^7 thiol was proposed to coordinate to the metal oxo center, along with the amide nitrogen, thioether and amine of the Apc^2 residue (a modified Cys containing a bidentate side chain).[13] The remaining Re-cyclized complexes exhibited NS_3 metal coordination, with three of the coordination sites filled by the Cys thiols, and the amide nitrogen between two adjacent Cys residues completing the coordination.[13]

In vitro stability was assessed for purified ^{99m}Tc -labeled TATE and derivatives **1–6** under physiological conditions (pH = 7.4 and 37 °C) in PBS (Table 2), mouse serum (Table 3), and 10 mM Cys solution (Table 4). The percentage of intact peptide was examined by RP-HPLC for aliquots of the incubation mixtures removed at 1, 2, 4 and 24 h. Replicate measurements were obtained by radio-TLC using two developing solutions. In 10% ammonium acetate (w/v), intact radiolabeled peptide remained at the origin, separating it from any $^{99m}\text{TcO}_4^-$ present, which moved near the solvent front ($R_f \sim 0.8$). In 1:1 solvent A:solvent B, both the radiolabeled peptide and any $^{99m}\text{TcO}_4^-$ present moved near the solvent front, allowing the absence of signal at the origin to confirm that no colloids

($^{99m}\text{TcO}_2$) had been formed. Stability measurements were performed in triplicate, and the percentages of radioactivity at the origin and at $R_f \sim 0.8$ are given in Tables 2–4.

Significant decomposition was observed for ^{99m}Tc -TATE in PBS, mouse serum, and 10 mM Cys challenge under physiological conditions, as expected from our earlier findings.[9] We previously proposed that the instability of ^{99m}Tc -TATE was due to an 8-membered ring formed by *N*-terminal amine coordination to the metal upon cyclization (Figure 1a), with loose coordination leaving the ^{99m}Tc vulnerable to oxidation and loss of the radiometal center as $^{99m}\text{TcO}_4^-$. Acetylating the *N*-terminus of the TATE peptide, in an attempt to disfavor *N*-terminal amine coordination to ^{99m}Tc and thereby improve stability, resulted in ^{99m}Tc -**1**, with stability marginally improved in PBS, slightly lowered in mouse serum, and equivalent in 10 mM cysteine challenge when compared with ^{99m}Tc -TATE. The similar in vitro performance of ^{99m}Tc -**1** to ^{99m}Tc -TATE may be attributed to 8-membered rings within each of the peptide structures (Figure 1b and 1a, respectively). Interestingly, when the cysteine solution concentration was lowered to 1 mM, the stability of ^{99m}Tc -**1** improved to a much larger degree than did ^{99m}Tc -TATE, showing that the *N*-acetylation of the TATE peptide did result in improved protection of the metal center.

Increasing the number of cysteines in a peptide sequence has proven an effective strategy to enhance stability in other peptide systems. For example, Giblin et al. reported the addition of a third Cys to an alpha-melanocyte-stimulating hormone (α -MSH) peptide, which resulted in a marked improvement in stability as well as in receptor-binding affinity.[15] Therefore, four peptides (**2** through **5**) were designed to incorporate three Cys residues to further improve stability of the metal-cyclized peptides by strengthening the radiometal coordination and resulting in more favorable 5- and 6-membered ring sizes. Additionally, a final peptide (**6**) was designed in an effort to yield an N_2S_2 radiometal coordination mode with stable ring sizes. Structural information on the non-radioactive Re analogues has been published, and based on co-elution of the ^{99m}Tc analogues with their Re counterparts (Figure 1) we presume they are very similar.[13,14] Each of the three-Cys ^{99m}Tc -cyclized TATE derivatives (^{99m}Tc -**2** through ^{99m}Tc -**5**) demonstrated similar (middle to upper 90% range) in vitro stability out to 24 h in the various solutions tested under physiological conditions and were the greatest observed in the series. The stability of ^{99m}Tc -**6** was markedly improved over ^{99m}Tc -TATE and ^{99m}Tc -**1**, but was not as stable as ^{99m}Tc -**2** through ^{99m}Tc -**5**, with intact peptide percentages at 24 h in the low to middle 80s probably due to the less favorable thioether coordination.

While the modified TATE peptide sequences reported herein led to improvements in stability at the radiotracer level, the Re-cyclized counterparts were previously shown to have moderate to poor SSTR binding affinities. The IC_{50} values for Re-TATE and Re-**1** were 29 nM and 106 nM, respectively,[14] and the Re-cyclization products of compounds **2** through **6** had micromolar and higher IC_{50} values.[13] Though the TATE peptide sequence stood out as the clear lead in our early studies, low and non-selective in vivo uptake of ^{99m}Tc -TATE in SSTR rich tissues (e.g., pancreas and adrenals) and SSTR2-expressing AR42J tumors, with evidence of radiotracer instability (e.g., uptake in stomach and thyroid), was observed. [9]

To probe the interplay between improvements in radiotracer stability and losses in SSTR2 affinity and its effects on SSTR targeting in vivo, two ^{99m}Tc -TATE derivatives were selected for in vivo studies: ^{99m}Tc -**1** (slightly better stability than ^{99m}Tc -TATE with slightly worse SSTR2 affinity) and ^{99m}Tc -**2** (greatly improved stability with greatly reduced SSTR2 affinity). The results of normal female CF-1 mouse biodistribution studies for ^{99m}Tc -**1** and ^{99m}Tc -**2** are shown in Tables 5 and 6, respectively, with a similar study for ^{99m}Tc -TATE [9] shown in Table 7 for comparison.

The biodistribution data for all three ^{99m}Tc -cyclized peptides indicated low uptake in the SSTR-expressing target tissues (pancreas and adrenals), which was not blocked by co-administration of non-radioactive disulfide-cyclized Tyr³-octreotate. Interestingly, the biodistribution data for ^{99m}Tc -TATE and ^{99m}Tc -**2**, which differed greatly by in vitro stability, were nearly the same. The uptake in the various tissues and organs for ^{99m}Tc -**1** were approximately two to four times higher than TATE and ^{99m}Tc -**2**, perhaps due to the higher initial amount of the compound in the blood and the longer blood pool circulation.

Uptake in the stomach and significant intestinal clearance was also observed in all three cases, potentially as a result of radiotracer instability and/or from actual targeting of somatostatin receptors within the stomach. However, the low uptake in the pancreas and adrenals suggests instability of the peptides over targeting of the stomach. It is possible that ^{99m}Tc -**2** is unstable in vivo despite being quite stable under the various in vitro conditions tested herein. From these data alone, it is difficult to discern whether the poor in vivo performance of ^{99m}Tc -**2** is due to its low SSTR2 affinity or to instability. In either case, the low and non-selective uptake of all three ^{99m}Tc -peptides in somatostatin receptor-expressing tissues precludes their use as radiodiagnostic agents.

4. Conclusions

Tyr³-octreotate and six variations of its peptide sequence were cyclized with ^{99m}Tc and evaluated in vitro and in vivo for stability and targeting. The incorporation of a third Cys to yield an NS₃ coordination motif in the elongated peptides **2** through **5** provided the greatest stability ^{99m}Tc -peptide complexes. No significant differences were observed in normal CF-1 mouse biodistribution studies between ^{99m}Tc -TATE and ^{99m}Tc -**1** and ^{99m}Tc -**2**, suggesting that the lowered SSTR2 affinities in these peptides outweighed their improvements in radiotracer stability. Since the biological performance was not significantly improved, rigorous studies to further characterize the ^{99m}Tc peptide structures and to confirm the proposed coordination motifs were not performed. Finding synergy between stabilization of the ^{99m}Tc metal center and affinity for the somatostatin receptor remains a challenge yet to be solved. Computational studies are underway to determine the factors most important to complex stability and receptor binding affinity.

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Figure 1.

The structures of the Re-cyclization products of TATE (a), compound **1** (b), compound **2** (c), and compound **6** (d)[9,13].

Table 1Sequences of Tyr³-octreotate derivatives and Sst Receptor Binding Affinities of their Re-cyclized products

Derivative	Sequence	IC ₅₀ of Re-peptides	Reference
TATE	DPhe-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)	29.2 ± 6.2 nM	[14]
1	Ac-DPhe-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)	106 ± 12 nM	[14]
2	Ac-Cys-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)	3.4 ± 0.9 μM	[13]
3	Ac-DCys-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)	>100 μM	[13]
4	Ac-DPhe-Cys-Cys-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)	>100 μM	[13]
5	Ac-DPhe-Cys-Tyr-DTrp-Lys-Thr-Cys-Cys-Thr(OH)	>100 μM	[13]
6	Ac-DPhe-Apc-Tyr-DTrp-Lys-Thr-Cys-Thr(OH)	>100 μM	[13]

Table 2

Stability of ^{99m}Tc -cyclized analogues in PBS^a

Time	^{99m}Tc -cyclized Analogue					
	TATE	1	2	3	4	5
1 h	86.2 ± 5.4	90.2 ± 2.4	98.3 ± 0.5	99.5 ± 0.2	99.3 ± 0.4	98.5 ± 0.8
2 h	73.7 ± 4.3	83.4 ± 5.2	98.0 ± 0.2	99.0 ± 0.8	99.6 ± 0.1	97.8 ± 0.7
4 h	60.4 ± 1.8	76.3 ± 3.4	97.8 ± 0.9	98.5 ± 0.7	99.6 ± 0.2	98.5 ± 0.8
24 h	37.9 ± 7.7	52.0 ± 5.6	94.1 ± 1.4	96.1 ± 1.0	95.8 ± 2.7	86.9 ± 6.9
						82.3 ± 2.7

^aStudies were performed in PBS at physiological conditions (pH = 7.4 and 37 °C). Data are presented as percent intact ^{99m}Tc -analogue post purification.

Table 3

Stability of ^{99m}Tc -cyclized analogues in serum^a

Time	^{99m}Tc -cyclized Analogue					
	TATE	1	2	3	4	5
1 h	97.4 ± 1.9	91.4 ± 1.8	96.7 ± 0.8	99.0 ± 0.8	99.2 ± 0.3	94.2 ± 0.5
2 h	97.1 ± 1.5	81.7 ± 4.1	97.1 ± 0.7	99.4 ± 0.2	99.5 ± 0.1	95.1 ± 0.9
4 h	95.2 ± 1.7	81.4 ± 4.7	97.0 ± 0.2	97.8 ± 1.8	99.7 ± 0.1	94.5 ± 1.9
24 h	57.6 ± 7.5	51.2 ± 4.4	94.7 ± 2.4	93.5 ± 1.7	98.9 ± 0.9	86.6 ± 6.1

^aStudies were performed in mouse serum at physiological conditions (pH = 7.4 and 37 °C). Data are presented as percent intact ^{99m}Tc -analogue post purification.

Table 4

Stability of ^{99m}Tc -cyclized analogues to cysteine challenge

Time	^{99m} Tc-cyclized Analogue								
	TATE ^a	1 ^a	2 ^a	3 ^a	4 ^a	5 ^a	6 ^a	TATE ^b	1 ^b
1 h	63.9 ± 4.1	60.1 ± 5.1	99.4 ± 0.3	99.2 ± 0.5	99.0 ± 0.4	99.5 ± 0.4	98.8 ± 0.7	95.8 ± 2.4	97.1 ± 3.3
2 h	53.3 ± 3.5	55.8 ± 6.9	99.1 ± 0.5	99.5 ± 0.1	98.7 ± 0.3	99.4 ± 0.2	98.8 ± 0.9	85.3 ± 4.0	95.1 ± 2.7
4 h	42.5 ± 5.6	38.9 ± 3.7	99.3 ± 0.1	99.6 ± 0.2	99.2 ± 0.5	99.4 ± 0.1	98.0 ± 0.9	77.8 ± 3.3	87.2 ± 1.0
24 h	15.0 ± 8.3	22.9 ± 4.5	93.3 ± 0.7	97.1 ± 0.8	94.0 ± 5.0	99.2 ± 0.5	93.7 ± 9.6	45.0 ± 5.8	81.7 ± 1.8

Studies were performed in 10 mM Cys^a or 1 mM Cys^b at physiological conditions (pH = 7.4 and 37 °C). Data are presented as percent intact ^{99m}Tc -analogue post purification.

Table 5In vivo biodistribution (%ID/g) of ^{99m}Tc -Ac-Tyr³-octreotate (^{99m}Tc -1) in normal CF-1 mice

Tissue/Organ	1 h	1 h block	4 h	24 h
Blood	3.81 ± 0.10	4.31 ± 0.44	1.50 ± 0.29	0.15 ± 0.02
Liver	4.17 ± 0.34	6.47 ± 1.64	1.58 ± 0.06	0.39 ± 0.03
Spleen	1.30 ± 0.17	1.74 ± 0.24	0.55 ± 0.13	0.08 ± 0.01
Kidney	12.05 ± 2.14	8.00 ± 1.74	6.54 ± 0.23	2.04 ± 0.37
Muscle	0.53 ± 0.06	0.68 ± 0.06	0.25 ± 0.06	0.05 ± 0.02
Stomach	11.69 ± 10.10	14.18 ± 4.97	7.54 ± 4.16	1.26 ± 0.49
Sm. Int	15.48 ± 3.35	10.91 ± 2.12	2.02 ± 0.67	0.68 ± 0.20
Lg. Int	2.55 ± 0.21	1.70 ± 0.18	22.09 ± 4.99	3.08 ± 1.60
Adrenals	1.53 ± 0.30	2.18 ± 0.69	0.86 ± 0.43	0.12 ± 0.03
Pancreas	1.49 ± 0.06	2.08 ± 0.31	0.55 ± 0.04	0.07 ± 0.01

Data presented as average ± SD; n = 5 for 1 h and 24 h; n = 4 for 1 h block and 4 h.

Table 6In vivo biodistribution (%ID/g) of ^{99m}Tc -Ac-Cys¹,Tyr³-octreotate (^{99m}Tc -2) in normal CF-1 mice

Tissue/Organ	1 h	1 h block	4 h	24 h
Blood	0.95 ± 0.12	1.38 ± 0.15	0.34 ± 0.04	0.06 ± 0.01
Liver	1.60 ± 0.17	1.78 ± 0.59	0.47 ± 0.15	0.11 ± 0.01
Spleen	0.38 ± 0.09	0.47 ± 0.07	0.12 ± 0.01	0.04 ± 0.01
Kidney	2.61 ± 0.64	2.97 ± 0.19	1.43 ± 0.34	0.48 ± 0.10
Muscle	0.26 ± 0.02	0.43 ± 0.05	0.08 ± 0.04	0.01 ± 0.01
Stomach	2.95 ± 1.42	5.69 ± 0.45	3.88 ± 0.54	0.61 ± 0.15
Sm. Int	20.28 ± 3.58	24.29 ± 3.21	1.94 ± 0.50	0.22 ± 0.04
Lg. Int	0.53 ± 0.08	0.56 ± 0.04	28.18 ± 4.57	1.18 ± 0.66
Adrenals	0.36 ± 0.04	0.63 ± 0.19	0.22 ± 0.07	0.03 ± 0.01
Pancreas	0.46 ± 0.15	0.54 ± 0.10	0.15 ± 0.03	0.02 ± 0.01

Data presented as average ± SD; n = 5 for 1 h, 4 h, and 24 h; n = 4 for 1 h block.

Table 7In vivo biodistribution (%ID/g) of ^{99m}Tc -Tyr³-octreotate (^{99m}Tc -TATE) in normal CF-1 mice [9]

Tissue/Organ	1 h	1 h block	4 h	24 h
Blood	0.96 ± 0.01	2.39 ± 1.91	0.44 ± 0.05	0.05 ± 0.01
Liver	1.09 ± 0.13	1.69 ± 0.28	0.66 ± 0.03	0.22 ± 0.03
Spleen	0.36 ± 0.05	0.46 ± 0.10	0.20 ± 0.02	0.05 ± 0.01
Kidney	2.24 ± 0.16	3.61 ± 0.59	1.37 ± 0.29	0.60 ± 0.05
Muscle	0.11 ± 0.01	0.12 ± 0.01	0.05 ± 0.01	0.01 ± 0.01
Stomach	2.73 ± 0.35	2.65 ± 0.20	4.82 ± 0.95	0.69 ± 0.23
Sm. Int	28.53 ± 4.04	33.21 ± 4.06	2.08 ± 1.08	0.19 ± 0.01
Lg. Int	1.63 ± 0.78	0.51 ± 0.11	60.20 ± 6.39	0.59 ± 0.24
Adrenals	0.24 ± 0.09	0.25 ± 0.02	0.14 ± 0.06	0.01 ± 0.01
Pancreas	0.38 ± 0.13	0.60 ± 0.18	0.19 ± 0.06	0.03 ± 0.01

Data presented as average ± SD; n = 5 for 1 h; n = 4 for 1 h block, 4 h and 24 h.