^{99m}Tc-HYNIC-[Tyr^3]-Octreotide for Imaging Somatostatin-Receptor–Positive Tumors: Preclinical Evaluation and Comparison with ^{111}In-Octreotide

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In this paper we describe the preclinical evaluation of ^{99m}Tc-hydrazinonicotinyl-Tyr^3-octreotide (HYNIC-TOC) using different coligands for radiolabeling and a comparison of their in vitro and in vivo properties with ^{111}In-diethylenetriaminepentaacetic acid (DTPA)-octreotide. **Methods:** HYNIC-TOC was radiolabeled at high specific activities using tricine, ethylenediaminediacetic acid (EDDA), and tricine–nicotinic acid as coligand systems. Receptor binding was tested using AR42J rat pancreatic tumor cell membranes. Internalization and protein binding studies were performed, and biodistribution and tumor uptake were determined in AR42J tumor-bearing nude mice. **Results:** All ^{99m}Tc-labeled HYNIC peptides showed retained somatostatin-receptor binding affinities (K_d < 2.65 nM). Protein binding and internalization rates were dependent on the coligand used. Specific tumor uptake between 5.8 and 9.6 percentage injected dose per gram (%ID/g) was found for the ^{99m}Tc-labeled peptides, compared with 4.3 %ID/g for ^{111}In-DTPA-octreotide. Tricine as coligand showed higher activity levels in muscle, blood, and liver, whereas tricine–nicotinic acid produced significant levels of activity in the gastrointestinal tract. EDDA showed the most promising overall biodistribution profile, with tumor-to-liver and tumor-to-gastrointestinal tract ratios similar to those obtained with ^{111}In-DTPA-octreotide, lower ratios in blood and muscle, but considerably higher tumor-to-kidney ratios. **Conclusion:** TOC can be radiolabeled to high specific activities using HYNIC as a bifunctional chelator. The high specific tumor uptake, rapid blood clearance, and predominantly renal excretion make ^{99m}Tc-EDDA-HYNIC-TOC a promising candidate for an alternative to ^{111}In-DTPA-octreotide for tumor imaging.

Key Words: somatostatin; octreotide; hydrazinonicotinamide; ^{99m}Tc

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Over the last few years, ^{111}In-diethylenetriaminepentaacetic acid (DTPA)-octreotide has found widespread clinical applicability, especially in oncology (*1–3*). However, limitations, especially concerning availability, imaging properties, and costs, remain and have stimulated research on radiolabeling with many alternative radionuclides, including ^{90}Y and ^{161}Tb (*4*) for therapeutic applications; ^{68}Ga (*5*), ^{64}Cu (*6*), and ^{18}F (*7*) for positron imaging; and, in particular, ^{99m}Tc. Thus, ^{99m}Tc labeling of somatostatin analogues has been extensively studied, including attempts at direct labeling after reduction of the disulfide bridge (*8,9*) and the use of bifunctional chelators such as propyleneaminoxime (*10*), triamidomonothiols (*11*), and tetramines (*12,13*). One analogue based on a carbocyclic peptide and a ^{99m}Tc-N_3S-chelating moiety (P829) has been studied extensively in clinical trials (*14*). Recently, Maecke and Behe (*15*) described the use of the hydrazinonicotinyl (HYNIC) core to prepare ^{99m}Tc-labeled octreotide derivatives with binding affinity to somatostatin receptors and identified [Tyr^3]-octreotide as a particularly promising analogue. We have therefore evaluated this labeling approach in some detail (*16,17*) using different coligands based on aminocarboxylates (*18*) and tricine ternary ligand systems (*19*). In this article, we describe the preclinical evaluation of ^{99m}Tc-[HYNIC-Tyr^3]-octreotide (HYNIC-TOC) labeled through 3 different coligands: tricine, ethylenediaminediacetic acid (EDDA), and a tricine–nicotinic acid ternary ligand system, including a comparison of the biodistribution of the ^{99m}Tc-labeled peptides with that of ^{111}In-DTPA-octreotide.

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MATERIALS AND METHODS

Peptides

Tyr^3-octreotide (TOC) [H-(D)Phe-Cys-Tyr-(D)Trp-Lys-Thr-Cys-Thr(ol)] and Lys^5(BOC)-TOC (where BOC is butoxycarbonyl) were purchased from Bachem Ltd., Saffron Walden, UK.

HPLC

A solvent module 125 (Beckman Coulter, Inc., Fullerton, CA) with a 166-nm ultraviolet detector (Beckman) and radiometric detection was used for reverse-phase high-performance liquid chromatography (HPLC) analysis and preparation. An Ultrasphere ODS 5-μm system (Beckman), 4.6 × 250 mm column, 1 mL/min flow rate, and 220-nm ultraviolet detection, was used with the following solvent systems: method 1—acetonitrile (ACN):0.1% trifluoroacetic acid:water (0–3 min, 0% ACN; 3–10 min, 0–40% ACN; 10–20 min, 40% ACN; 20–23 min, 40–70% ACN; 26–27

min, 70–100% ACN) or method 2—ACN:0.01 N phosphate buffer, pH 6.2 (0–3 min, 0% ACN; 3–10 min, 0–25% ACN; 10–20 min, 25% ACN; 20–23 min, 25–70% ACN; 26–27 min, 70–100% ACN).

SPE Purification

For purification of the radiolabeled peptide for stability and protein binding studies, a solid-phase extraction (SPE) method was used. The radiolabeling mixture was passed through a preactivated C_{18} SepPak mini cartridge (Waters, Milford, MA). The cartridge was washed with 5 mL water, the radiolabeled peptide was eluted with 80% acetonitrile, and the organic solvent was evaporated under a vacuum. This method efficiently removed all hydrophilic impurities ($^{99m}TcO_4^-$, ^{99m}Tc-coligand) and ^{99m}Tc-colloid to a concentration of less than 2% when tested by HPLC or thin-layer chromatography.

Synthesis of HYNIC-TOC

Five micromoles 6-BOC-hydrazinopyridine-3-carboxylic acid (BOC-HYNIC), 6 μmol O-(7-azabenzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate, and 20 μmol diisopropylethylamine in 300 μL dimethylformamide were allowed to react for 15 min at room temperature. Sixty microliters of this solution were added to 1 μmol [Lys^5-BOC]-protected TOC (Bachem) in a mixture of 20 μl dimethylformamide (DMF):5 μL water and allowed to react for 1 h. The resulting solution was purified on a SepPak column, and the peptide was deprotected with trifluoroacetic acid containing 2% thioanisole and purified on HPLC using method 1.

Radiolabeling of HYNIC-TOC

Tricine as Coligand. In a rubber-sealed vial, 10 μg HYNIC-TOC were incubated with 0.5 mL tricine solution (100 mg/mL in 25 mmol/L succinate buffer, pH 5.0), 0.5 mL $^{99m}TcO_4^-$ solution (100–1000 MBq), and 25 μL tin(II) solution (10 mg $SnCl_2 \cdot 2H_2O$ in 10 mL nitrogen-purged 0.1 N HCl) for 30 min at room temperature.

EDDA as Coligand. Ten micrograms HYNIC-TOC were incubated with 0.5 mL EDDA solution (10 mg/mL, pH 7.0), 0.5 mL $^{99m}TcO_4^-$ solution (100–1000 MBq), and 5–10 μL tin(II) solution (10 mg $SnCl_2 \cdot 2H_2O$ in 10 mL nitrogen-purged 0.1 N HCl) for 60 min at room temperature.

Tricine and Nicotinic Acid as Coligands (Ternary Coligand System). Ten micrograms HYNIC-TOC, 0.4 mL tricine solution (100 mg/mL in 25 mmol/L succinate buffer, pH 5.0), 0.1 mL nicotinic acid (20 mg/mL in 25 mmol/L succinate buffer, pH 5.0), 0.5 mL $^{99m}TcO_4^-$ solution (100–1000 MBq), and 25 μL tin(II) solution (10 mg $SnCl_2 \cdot 2H_2O$ in 10 mL nitrogen-purged 0.1 N HCl) were heated for 15 min at 100°C.

^{111}In-DTPA-octreotide was prepared from a commercial kit (Octreoscan; Mallinckrodt Medical BV, Petten, The Netherlands).

Plasma Protein Binding

Protein binding of the SepPak-purified ^{99m}Tc-labeled peptide was determined after 15 min and 1, 2, 3, and 4 h of incubation in human plasma at 37°C using size exclusion chromatography with Sephadex G-50 minicolumns (Microspin G-50; Pharmacia Biotech, Piscataway, NJ). The columns were prespun at 2000*g* for 1 min. A 25-μl plasma sample was added, and the column was centrifuged again at 2000*g* for 2 min. The collected eluate and the column were counted in an NaI scintillation counter, and protein-bound peptide was calculated as the percentage eluted from the column. Controls were studied after incubation in phosphate-buffered saline for 1 h at 37°C.

Somatostatin Receptor Binding

The binding affinity of peptide conjugates was tested in a competition assay against ^{125}I-somatostatin-14 as described previously (*20*). Rat pancreatic tumor cell (AR42J) membranes were used as a source for somatostatin receptors, bound radioligand was separated from free radioligand by filtration through glass fiber filters (grade GF/C; Whatman, Fairfield, NJ), and inhibitory concentration of 50% (IC_{50}) values were calculated using nonlinear regression with version 5.0 Origin software (Microcal, Northampton, MA). The specific binding of the ^{99m}Tc-labeled peptides was determined by competition against unmodified TOC in a similar assay. K_d values of carrier-free ^{99m}Tc-labeled peptide conjugates (HPLC-purified, method 2) were determined in radioligand saturation assays using AR42J cell membranes as the receptor source with increasing amounts of peptide and 1 μmol/L cold peptide to determine nonspecific binding.

Internalization Studies

Studies of the internalization of receptor-bound ^{99m}Tc-labeled peptides were performed by adapting methods described previously (*21,22*). Live AR42J cells were washed twice with culture medium (Roswell Park Memorial Institute 1640/2% bismuth-sulfite agar). Cells equivalent to 0.5 mg protein were incubated in triplicate with 150,000-cpm carrier-free ^{99m}Tc-labeled peptide (HPLC-purified, method 2) with and without an excess of cold peptide (1 μmol/L) at 37°C. After 20, 40, 60, and 90 min, cells were separated by centrifugation and washed with cold culture medium. Surface-bound activity was removed by incubation with acid buffer (50 mmol/L glycine-HCl/100 mmol/L NaCl, pH 2.8) at room temperature for 20 min, followed by 2 washing steps. Surface-bound activity and internalized activity were measured and related to the total activity added.

In Vivo Tumor Uptake Studies

Animal experiments were carried out in compliance with the Animals (Scientific Procedures) Act 1995, according to British law.

AR42J rat pancreatic tumor cells ($5–10 \times 10^6$ cells) were injected subcutaneously into the flank of nu/nu mice (Imperial Cancer Research Fund, London, UK). Between 10 and 30 days later, tumors had grown to a size of 0.5–1 mL. Eight animals were studied simultaneously, 4 of them with intraperitoneal pretreatment of 50 μg octreotide 30 min before injection of the radiopharmaceutical. The animals were killed 4 h after 1 MBq ^{99m}Tc-labeled peptide was injected into the tail vein, and samples of different organs and the tumor were dissected and counted. Uptake of the radiopharmaceutical in terms of percentage injected dose (%ID)/g and %ID/organ was calculated by reference to standards prepared from dilutions of the injected preparation. Specific uptake was determined by comparison of blocked and unblocked animals using the Student *t* test.

RESULTS

Radiolabeling, In Vitro Stability, and Protein Binding

Radiolabeling of HYNIC-TOC was performed at specific activities greater than 37 GBq/μmol. Quantitative labeling (>98%) was achieved with tricine and tricine–nicotinic acid as coligand, whereas with EDDA as coligand an average labeling yield of 63% was observed (Table 1). The resulting ^{99m}Tc complexes showed a high in vitro stability. Labeling

TABLE 1
In Vitro Data for ^{99m}Tc-labeled HYNIC-TOC and Nonconjugated TOC

	IC_{50} of cold conjugates (nmol/L)	Coligand	Labeling yield (%)	K_d of ^{99m}Tc-peptides (nmol/L)
HYNIC-TOC	0.65	Tricine	98.2	1.14
		Tricine–nicotinic acid	98.7	2.11
		EDDA	63.6	2.65
TOC	0.50	—	—	—

and stability studies have been described in greater detail elsewhere (*16*).

Plasma protein binding depended on the coligand used. Results of time-course studies are shown in Figure 1. When tricine was used as coligand, an increase in protein binding over time from 12% to greater than 30% was observed. Using the ternary coligand system tricine–nicotinic acid, a steady level of protein binding of approximately 17% was achieved. EDDA showed the lowest level of protein binding—less than 10%—with no significant increase over time.

Receptor Binding and Internalization

The results of receptor binding studies are summarized in Table 1. In displacement studies using ^{125}I-somatostatin-14 as the radioligand, an IC_{50} value for HYNIC-TOC of 0.6 nmol/L, compared with 0.5 nmol/L for unmodified TOC, was determined. Displacement studies with TOC using the ^{99m}Tc-labeled peptide as radioligand showed specific binding for all preparations independent of the coligand used. Saturation studies with the ^{99m}Tc-labeled peptide HYNIC conjugates resulted in K_d values in the nmol/L range: 1.1 nmol/L for tricine, 2.1 nmol/L for the tricine–nicotinic acid ternary complex, and 2.6 nmol/L for EDDA as coligand. Figure 2 shows binding curves from displacement studies and saturation assays with corresponding Scatchard analysis for EDDA and tricine–nicotinic acid as coligand.

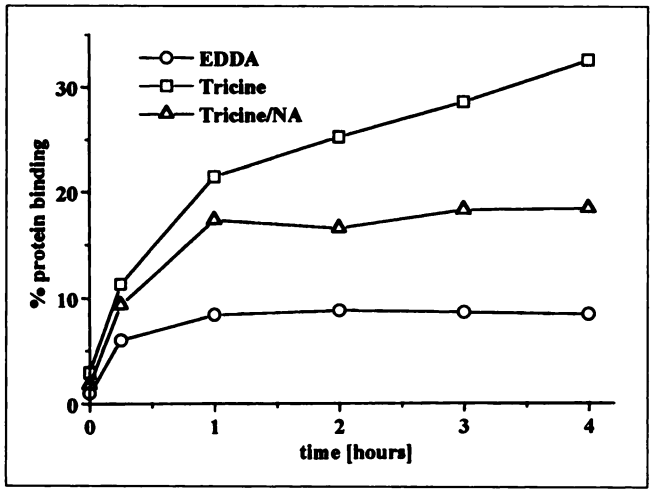

FIGURE 1. Protein binding versus time after ^{99m}Tc-HYNIC-TOC injection. NA = nicotinic acid.

Receptor binding studies on intact cells showed a rapid internalization of the ^{99m}Tc-labeled peptides. Figure 3 shows the internalization of ^{99m}Tc-HYNIC-TOC using EDDA and tricine–nicotinic acid as coligands. Surface-bound activity remained less than 1% per milligram of protein, and internalized activity rapidly increased over time, with a faster internalization for EDDA (5% per milligram of protein after 80 min) compared with tricine–nicotinic acid as coligand (2% per milligram of protein after 80 min). With tricine alone as coligand, a high nonspecific binding was observed, and no quantitative comparison could be made.

Biodistribution and Tumor Uptake

Biodistribution data of ^{99m}Tc-labeled HYNIC-TOC using different coligands in AR42J tumor-bearing nude mice in comparison with ^{111}In-DTPA-octreotide are summarized in Table 2. Significant differences (*t* test, $P < 0.05$) in tumor uptake between blocked and unblocked animals were found for all compounds tested. A higher specific, but also nonspecific, tumor uptake was found for all ^{99m}Tc-labeled peptides (EDDA as coligand, 9.6 and 1.8 %ID/g, respectively; tricine as coligand, 9.6 and 3.0 %ID/g, respectively; and tricine–nicotinic acid as coligand, 5.8 and 1.0 %ID/g, respectively) compared with ^{111}In-DTPA-octreotide (4.3 and 0.8 %ID/g, respectively).

The highest residual activity levels in all organs were found for tricine as coligand, especially in blood (1.14 %ID/g), muscle (0.92 %ID/g), and liver (2.08 %ID/g). EDDA and tricine–nicotinic acid as coligands showed higher activity levels compared with ^{111}In-DTPA-octreotide in blood (>0.26 versus 0.07 %ID/g), liver (>0.75 versus 0.47 %ID/g), and gut (>1.58 versus 0.55 %ID/g) but considerably lower levels in kidneys (<4.7 versus 22.1 %ID/g). EDDA showed higher levels in muscle, liver, and spleen compared with tricine–nicotinic acid but lower levels in gut.

Tumor-to-organ ratios are also shown in Table 2. The lowest ratios were found for tricine as coligand. ^{111}In-DTPA-octreotide showed the highest ratios, especially for blood (62.5) and muscle (52.3); tumor-to-liver (9.14) and tumor-to-gastrointestinal tract (7.7) ratios were similar to those with EDDA as coligand (9.11 and 6.11, respectively). Tricine–nicotinic acid showed high tumor-to-muscle ratios (51.14) but low tumor-to-gastrointestinal tract ratios (1.78). Kidney uptake was considerably lower for EDDA and tricine–nicotinic acid compared with ^{111}In-DTPA-octreotide, with tumor-to-kidney ratios of 2.05, 1.59, and 0.19, respectively.

DISCUSSION

Our aim was to prepare a ^{99m}Tc-labeled analogue that is at least comparable with ^{111}In-DTPA-octreotide in its ability to image somatostatin receptors in vivo. Such an aim requires preparation of a ^{99m}Tc tracer with good in vitro and in vivo stability, high affinity for somatostatin receptors, and a favorable pattern of biodistribution. This last parameter

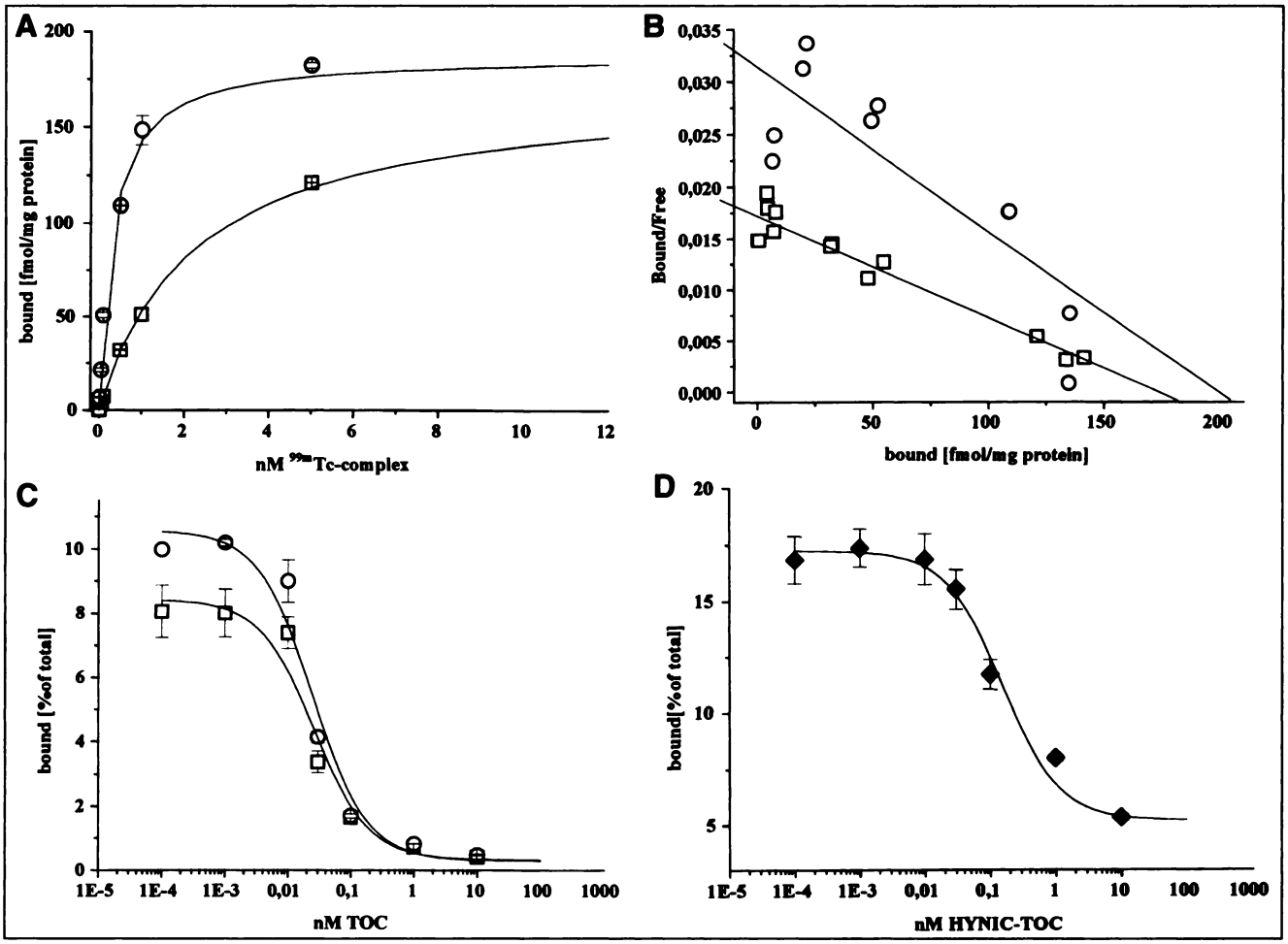

FIGURE 2. Receptor binding of ^{99m}Tc-labeled peptides on AR42J cell membranes. (A) Saturation curves of HYNIC-TOC with EDDA and HYNIC-TOC with tricine–nicotinic acid. (B) Scatchard plots corresponding to saturation curves. (C) Displacement curves of HYNIC-TOC with EDDA and HYNIC-TOC with tricine–nicotinic acid. Competitor is unmodified TOC. (D) Displacement curve of ^{125}I-somatostatin-14 using HYNIC-TOC as competitor.

requires that the compound show a rapid blood clearance and low uptake in receptor-negative tissues and organs of excretion. In particular, the radiopharmaceutical should be excreted predominantly through the renal system to avoid accumulation in the gastrointestinal tract and the consequent hindrance of detection of pelvic tumor deposits. In previous studies, we showed that both the peptide sequence and the bifunctional chelate system can profoundly influence the biodistribution pattern and excretory route (*17*) and that this in vivo behavior can be predicted somewhat by in vitro measurement of parameters such as stability, lipophilicity, and protein binding (*23*). In the studies, HYNIC showed a number of advantages over ^{99m}Tc labeling approaches using N_3S-based ligands as bifunctional chelators for labeling of small peptides for tumor imaging (*17*). In the current investigation, we have shown that HYNIC-TOC can be labeled at high specific activities (>37 GBq/μmol), resulting in stable complexes with retained binding affinity to somatostatin receptors in the nmol/L range. This finding agrees well with the findings of Maecke and Behe (*15*), who also showed that derivatization with spacers between the bifunctional chelator and the peptide does not improve the imaging properties of octreotide derivatives. The selection of coligands for use in the labeling is an important part of preclinical evaluation. Babich and Fischman (*24*) first showed that the nature of the coligand can influence the biologic properties of the radioconjugate, whereas Liu et al. (*18,19*) described an increased complex stability with EDDA and ternary ligand systems using *N*-heterocycles compared with tricine. The current study confirms that complex stability depends on the coligand used and also verifies our previous observation that patterns of biodistribution can be predicted in part by the plasma protein binding behavior. Thus, the use of tricine as a coligand showed the highest degree of plasma protein binding, corresponding with high levels of activity in blood, liver, spleen, and muscle. The 2 other coligands tested in this study showed much lower levels of protein binding, indicating a higher complex stability and correlating with considerably lower levels of activity, especially in blood and muscle. The ternary ligand system tricine–nicotinic acid showed some advantages in the labeling process in that it gave quantitative labeling

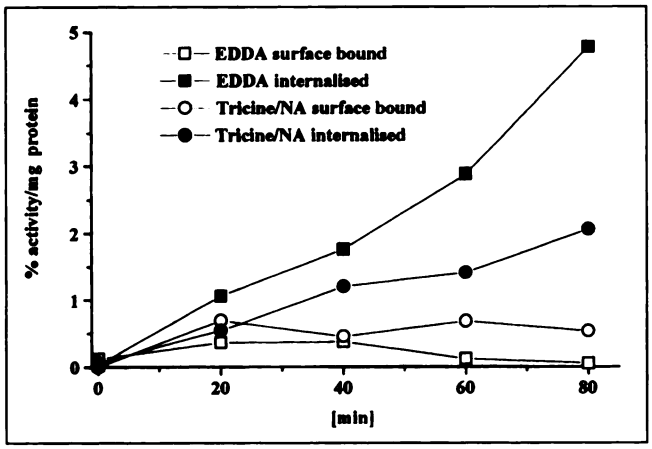

FIGURE 3. Specific internalization and surface binding versus time, as percentage of ^{99m}Tc-labeled peptides per milligram of protein (intact AR42J cells). NA = nicotinic acid.

yields, whereas EDDA typically gave only a 60% yield and required a purification step before use. The great improvement in complex stability compared with tricine alone indicates the potential of ternary coligand systems for ^{99m}Tc labeling for peptides in general, but the pattern of biodistribution for this complex was not ideal, with significant levels of activity appearing in the gastrointestinal tract. Despite a comparable in vitro binding affinity, tricine–nicotinic acid produced a lower tumor uptake (5.8 %ID/g) than did the other coligand systems (9.6 %ID/g for EDDA and tricine). A possible reason for the difference may be found in the relative rates of internalization of these compounds. In in vitro studies on AR42J cells, HYNIC-TOC with EDDA showed a significantly higher level of internalization than did the tricine–nicotinic acid conjugate (Fig. 3), comparable with levels observed for ^{111}In-labeled octreotide analogs (*22*). This increased rate of internalization of the radioligand–receptor complex, with subsequent retention of the radionuclide within the lysosomal compartment, seems likely to be in some degree responsible for the higher tumor retention 4 h after injection.

The use of EDDA as coligand produced the most promising pattern of biodistribution of all the ^{99m}Tc-TOC complexes explored, combining the highest degree of specific tumor uptake with the fastest blood clearance and the lowest levels of uptake in soft tissue and the gastrointestinal tract. In comparison with ^{111}In-DTPA-octreotide, a higher tumor uptake was seen, but also higher levels in some receptor-negative tissues, especially blood, muscle, liver, and spleen. HYNIC-TOC with EDDA thus produced tumor-to-organ ratios almost identical to those of ^{111}In-DTPA-octreotide in the liver and gastrointestinal tract, approximately half those of ^{111}In-DTPA-octreotide in blood and muscle, but 10-fold higher than those of ^{111}In-DTPA-octreotide in the kidney.

The question of how well HYNIC-TOC with EDDA will compare with octreotide in clinical studies remains to be answered, because studies in animal models can only partially predict the likely pattern of biodistribution in humans. However, some patient studies recently performed with ^{99m}Tc-tricine–HYNIC-TOC (*25*) have had promising results showing its ability to image somatostatin-positive tumors in humans. The faster clearance and lower soft-tissue and gastrointestinal uptake of the EDDA complex in animal studies raise hopes that its performance in humans will also be better than that of the tricine complex. The lower renal accumulation of ^{99m}Tc-labeled conjugates may also prove an

TABLE 2
Biodistribution and Tissue Ratios in AR42J Tumor-Bearing Nude Mice

	HYNIC-TOC with EDDA		HYNIC-TOC with tricine–nicotinic acid		HYNIC-TOC with tricine		^{111}In-octreotide	
Site	Unblocked	Blocked	Unblocked	Blocked	Unblocked	Blocked	Unblocked	Blocked
Mean ± SD								
Blood	0.28 ± 0.04	0.36 ± 0.06	0.26 ± 0.08	0.28 ± 0.03	1.14 ± 0.10*	1.31 ± 0.09	0.07 ± 0.01	0.06 ± 0.03
Liver	1.06 ± 0.40	0.99 ± 0.40	0.75 ± 0.67	0.42 ± 0.19	2.08 ± 0.40	2.20 ± 0.39	0.47 ± 0.09	0.47 ± 0.19
Kidney	4.71 ± 1.38	6.67 ± 3.05	3.64 ± 0.52	4.40 ± 0.75	14.57 ± 3.42	18.15 ± 5.34	22.12 ± 6.53	23.5 ± 14.39
Spleen	0.40 ± 0.15	0.31 ± 0.12	0.18 ± 0.04	0.18 ± 0.06	1.07 ± 0.16	0.98 ± 0.04	0.16 ± 0.02	0.19 ± 0.07
Pancreas	0.45 ± 0.23	0.26 ± 0.09	0.25 ± 0.03*	0.11 ± 0.02	1.66 ± 0.08*	0.80 ± 0.13	0.16 ± 0.07*	0.05 ± 0.02
Gut	1.58 ± 0.39	1.46 ± 0.73	3.25 ± 0.94	2.37 ± 0.51	2.32 ± 0.42	2.82 ± 0.86	0.55 ± 0.15	0.70 ± 0.50
Adrenals	0.86 ± 0.25*	0.43 ± 0.26	0.63 ± 0.23*	0.35 ± 0.08	1.80 ± 0.20*	1.24 ± 0.14	0.24 ± 0.07*	0.11 ± 0.08
Muscle	0.31 ± 0.28	0.41 ± 0.46	0.11 ± 0.08	0.08 ± 0.02	0.92 ± 0.14	0.73 ± 0.15	0.08 ± 0.07	0.03 ± 0.02
Tumor	9.65 ± 2.16*	1.82 ± 0.81	5.80 ± 2.31*	1.02 ± 0.19	9.58 ± 0.90*	3.04 ± 0.75	4.26 ± 1.00*	0.79 ± 0.25
Ratio								
Tumor to blood	33.97		22.67		8.38		62.54	
Tumor to liver	9.11		7.77		4.61		9.14	
Tumor to gut	6.11		1.78		4.13		7.68	
Tumor to muscle	31.29		51.14		10.46		52.28	
Tumor to kidney	2.05		1.59		0.66		0.19	

*Significant difference ($P < 0.05$) between blocked and unblocked animals.
Values are %ID/g 4 h after injection of ^{99m}Tc-labeled peptide, with blocking with 50 μg octreotide 30 min before injection.

advantage, because high kidney retention of ^{111}In-DTPA-octreotide is a significant problem in imaging this region.

Recently, another ^{99m}Tc-labeled somatostatin analogue, P829 (*26*) (depreotide), has been approved for human use. Depreotide is based on the carbocyclic structure of seglitide, which is known to have a different subtype specificity compared with octreotide (*27*). In clinical trials, depreotide has shown a pattern of tumor specificity different from that of octreotide, and a high binding affinity to somatostatin receptor subtype 3 has been found for P829 in a recent study (*28*). Depreotide itself cannot, therefore, be considered a direct substitute for octreotide. Because the amino acid sequence of Tyr^3-octreotide bears a closer resemblance to octreotide itself, HYNIC-TOC with EDDA and ^{111}In-DTPA octreotide might be expected to share a common profile of receptor subtype specificity, but this possibility remains to be proven.

CONCLUSION

We have shown that the hydrazinonicotinamide conjugate of Tyr^3-octreotide can be labeled with ^{99m}Tc to high specific activities and somatostatin binding affinity. EDDA was found to be the most promising of all the coligands tested.

Although the biodistribution of HYNIC-TOC with EDDA is different from that of ^{111}In-DTPA-octreotide, the high specific tumor uptake, low gastrointestinal activity, and rapid renal elimination with low renal retention make this ^{99m}Tc-labeled peptide a promising alternative to ^{111}In-DTPA-octreotide for imaging somatostatin receptor–positive tumors in humans. Clinical studies to explore this possibility are under way.

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