Preclinical Evaluation of Technetium-99m-Labeled Somatostatin Receptor-Binding Peptides

Shankar Vallabhajosula, Brian R. Moyer, John Lister-James, Bill J. McBride, Helena Lipszyc, Hiram Lee, Diago Bastidas and Richard T. Dean

Division of Nuclear Medicine, Department of Radiology, The Mount Sinai Medical Center, New York, New York; and Diatide, Inc., Londonderry, New Hampshire

We report here the results of studies on the in vitro receptor binding affinity, in vivo tumor uptake and biodistribution of two ^{99m}Tc-labeled peptides. **Methods:** Peptides P587 and P829 were synthesized by N-α-Fmoc peptide chemistry, purified by reversed-phase HPLC and characterized by fast-atom bombardment mass spectrometry. The peptides were labeled with ^{99m}Tc by ligand exchange from ^{99m}Tc-glucoheptonate. In vitro somatostatin receptors (SSTR)-binding affinities of P587, P829 and their oxorhenium complexes, [DTPA]octreotide and In-[DTPA]octreotide were determined in an inhibition assay using AR42J rat pancreatic tumor cell membranes and ^{125}I-[Tyr^3]somatostatin-14 as the probe. In vivo single- and dual-tracer studies of ^{99m}Tc peptides and ^{111}In-[DTPA]octreotide were carried out using Lewis rats bearing CA20948 rat pancreatic tumor implants. **Results:** Technetium-99m-P587 and ^{99m}Tc-P829 of high-specific activity (>60 Ci (2.2 TBq)/mmole) were prepared in >90% radiochemical yield. P587 and P829 had a Ki = 2.5 n*M* and 10 n*M*, respectively. [ReO]P587 and [ReO]P829, representing the ^{99m}Tc complexes, had Ki = 0.15 n*M* and 0.32 n*M*, respectively. In comparison, [DTPA]octreotide and In-[DTPA]octreotide had Ki = 1.6 and 1.2 n*M*, respectively. In vivo tumor uptake of ^{99m}Tc-P587 and ^{99m}Tc-P829 was high (4.1 and 4.9%ID/g at 90 min postinjection compared to 2.9% for ^{111}In-[DTPA]octreotide). Tumor/blood and tumor/muscle ratios at 90 min postinjection were 6 and 33 for ^{99m}Tc-P587, 21 and 68 for ^{99m}Tc-P829, and 22 and 64 for ^{111}In-[DTPA]octreotide. **Conclusion:** The high SSTR-binding affinity and high, receptor-specific and saturable in vivo tumor uptake indicate that ^{99m}Tc-P587 and ^{99m}Tc-P829 are promising radiotracers for the clinical detection of SSTR-expressing tumors and other tissues by ^{99m}Tc gamma scintigraphy.

Key Words: tumor imaging; somatostatin receptor imaging; technetium-99m; peptide; receptor binding

J Nucl Med 1996; 37:1016–1022

Natural somatostatin, also known as somatotropin release inhibiting factor (SRIF or SRIF-14), is a cyclic tetradecapeptide (Table 1) which is produced by the hypothalamus and pancreas and which, through binding to specific receptors and possibly through subsequent induced reduction in cellular cyclic AMP, inhibits the secretion of many hormones and growth factors (*2,3*). Receptors for SRIF have been found in the central nervous system, pituitary, pancreas and in the mucosa of the gastrointestinal tract. Five subtypes of human SRIF receptors, conventionally termed somatostatin-type receptors or SSTRs, hence SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5, have been cloned (*4–7*).

Most neuroendocrine tumors and their metastases express SSTRs to a much greater extent than do normal tissues (*8–11*). The types of tumors which have been found to express SSTRs include tumors of the amine-precursor-uptake-and-decarboxylation (APUD) cell system (APUDomas) such as small-cell lung cancer, endocrine pancreatic tumors, metastatic carcinoids, growth hormone-producing pituitary adenomas, paragangliomas, lymphomas (mainly Hodgkins), astrocytomas and meningiomas as well as some colorectal, breast and prostate cancers (as determined by ^{125}I-[Tyr^3]octreotide autoradiography (*9*)). SSTR2 appears to be the predominant SSTR sub-type expressed by these tumors (*10,11*).

SSTR-expressing tumors can be treated with SRIF or synthetic analogs to either reduce hypersecretion of hormones or inhibit tumor growth, or both (*8*). However, because SRIF undergoes rapid in vivo enzymatic degradation, SRIF analogs which are more resistant to in vivo degradation have been prepared (*12–16*). Octreotide (Sandostatin®; SMS 201-995) (Table 1) is a synthetic SRIF analog which is currently in clinical use for treating the hypersecretion of hormones symptomatic of gastroenteropancreatic (GEP) tumors, and acromegaly and is approved in the US for treatment of carcinoid tumors and VIPomas (*12*).

Lamberts and Krenning et al. (*1,8,17,18*) and Kvols et al. (*19*) have shown the radiolabeled octreotide derivatives, ^{123}I-[Tyr^3]octreotide and ^{111}In-[DTPA]octreotide, to be very useful for detecting small neuroendocrine tumors and metastases not detected by conventional means and for identifying tumors that respond to therapeutic doses of octreotide. Nevertheless, a ^{99m}Tc-labeled SSTR-binding radiotracer is highly desirable for routine nuclear medicine studies because ^{99m}Tc is considerably less expensive than ^{111}In and because ^{99m}Tc provides a greater photon flux, and hence better quality images, per unit of absorbed radiation dose.

We have developed a number of unique, high-affinity SSTR-binding peptides which can be radiolabeled readily with ^{99m}Tc with retention and, in many cases, enhancement of SSTR-binding affinity (*20*). Of these, ^{99m}Tc-P587 and ^{99m}Tc-P829 were selected for clinical studies and we describe their preclinical evaluation.

MATERIALS AND METHODS

Peptide Synthesis

Peptides P587, P829, octreotide and [DTPA]octreotide (see Table 1 for sequences) were synthesized at Diatide, Inc. (Londonderry, NH) using both solution and solid-phase peptide synthesis techniques and N-α-Fmoc chemistry. Details of the syntheses will appear elsewhere. The peptides were purified by preparative C_{18} reversed-phase HPLC using a Delta-Pak C_{18}, 15 μm, 300 Å, 47 × 300-mm column and 0.1% trifluoroacetic acid in water (0.1% TFA/H_2O) modified with 0.1% trifluoroacetic acid in 90% acetonitrile/10% water (0.1% TFA/(90% CH_3CN/H_2O)) as eluents, and then lyophilized. The purified peptides were lyophilized and their purity and identity were confirmed by analytical C_{18} reversed-

Received Jan. 19, 1995; revision accepted Nov. 3, 1995.

For correspondence or reprints contact: Shankar Vallabhajosula, PhD, Associate Professor of Radiology, Mount Sinai Medical Center, One Gustave L. Levy Pl., New York, NY 10029.

TABLE 1
Peptide Sequences*

Somatostatin: Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys
P587: cyclo-(N-Me)Phe-Tyr-(D-Trp)-Lys-Val-Hcy(CH_2CO-Gly-Gly-Cys-Lys-NH_2)
P829: cyclo-(N-Me)Phe-Tyr-(D-Trp)-Lys-Val-Hcy(CH_2CO-(β-Dap)-Lys-Cys-Lys-NH_2)
octreotide: (D-Phe)-Cys-Phe-(D-Trp)-Lys-Thr-Cys-Thr(ol)
[DTPA]octreotide: [DTPA]-(D-Phe)-Cys-Phe-(D-Trp)-Lys-Thr-Cys-Thr(ol)

*Cys ... Cys underline indicates cyclic disulfide; cyclo and underline indicates cyclic peptide.

Ala = L-alanine, Asn = L-asparagine, Cys = L-cysteine, β-Dap = β-(L-1,2-diaminopropionic acid), DTPA = diethylenetriaminepentaacetic acid, Gly = glycine, Hcy = L-homocysteine, Lys = L-lysine, (N-Me)Phe = N-α-methyl-L-phenylalanine, Phe = L-phenylalanine, D-Phe = D-phenylalanine, Ser = L-serine, Thr = L-threonine, Thr(ol) = L-threoninol, Trp = L-tryptophan, D-Trp = D-tryptophan, Tyr = L-tyrosine, Val = L-valine.

phase HPLC, C_{18}, 5 μm, 300 Å, 3.9 × 150-mm column and binary gradient elution with 0.1% TFA/H_2O as Solvent A and 0.1% TFA/(90% CH_3CN/H_2O) as Solvent B) and fast-atom bombardment mass spectrometry.

Peptide Metal Complexes

The oxorhenium complexes of P587 ([ReO]P587) and P829 ([ReO]P829) were prepared by ligand exchange using $Bu_4NReOBr_4$ (*21*) in DMF, followed by purification by preparative HPLC and confirmation of composition by electrospray mass spectrometry (ESMS). Indium-[DTPA]octreotide was prepared from In-chloride dissolved in 0.1 m citrate buffer at pH 5 and [DTPA]octreotide followed by purification by preparative HPLC and confirmation of composition by ESMS.

Preparation of Technetium-99m-Labeled Peptides

P587 and P829 were labeled with ^{99m}Tc by ligand exchange from ^{99m}Tc-glucoheptonate. P587 was dissolved to 1 mg/ml in 50% aqueous ethanol and P829 was dissolved to 1 mg/ml in normal saline. One quarter of a Glucoscan™ Kit (DuPont Pharma, N. Billerica, MA) that had been reconstituted with 1 ml ^{99m}Tc generator eluate (200–300 mCi; 7.4–11.1 GBq), was added to peptide solution. In the case of P587 the solution was heated at 100°C for 15 min. For P829, the reaction mixture was allowed to incubate at room temperature for 20 min. The radiochemical purity of the ^{99m}Tc-peptide complexes was determined by ITLC (ITLC-SG, Gelman Sciences, Ann Arbor, MI) developed in saturated saline (^{99m}Tc-peptide immobile, $^{99m}TcO_4$ and ^{99m}Tc-glucoheptonate mobile), ITLC-SG developed in 5:3:1.5 pyridine: acetic acid: water (^{99m}Tc-peptide, $^{99m}TcO_4$ and ^{99m}Tc-glucoheptonate mobile) and analytical reversed-phase HPLC, performed using an HPLC equipped with an in-line gamma detector linked to an integrating recorder, a Delta-Pak C_{18}, 5 μm, 300 Å, 3.9 × 150-mm column eluted at 1.2 ml/min with a gradient of 0.1% TFA/H_2O modified with 0.1% TFA/(90% CH_3CN/H_2O).

Preparation of Indium-111-[DTPA]Octreotide

Indium-111-[DTPA]octreotide was prepared by reacting ^{111}In-$InCl_3$ (1-2 mCi, 37-74 MBq) in 0.2 m HCl (1 ml) containing 25 mg trisodium citrate with 10 μg [DTPA]octreotide for 30 min at room temperature. The radiochemical purity of the ^{111}In-[DTPA]octreotide was determined by ITLC-SG developed in 0.1 m citrate buffer at pH 5.0.

In Vitro Assay

Peptides P587, P829 and [DTPA]octreotide and their metal complexes [ReO]P587, [ReO]P829 and In-[DTPA]octreotide were assayed in vitro for SSTR binding affinity by J.E. Taylor of Biomeasure, Inc., using AR42J rat pancreatic carcinoma cell membranes (expressing predominantly SSTR2 (*10*)) and ^{125}I-[Tyr^{11}]SRIF-14 as the probe. Briefly, AR42J cells were cultured in Dulbecco's Minimum Essential Medium supplemented with 10% fetal bovine serum and 8 mm L-glutamine maintained in a humidified 5% CO_2 atmosphere at 37°C in T-flasks. Harvested cells were homogenized in cold Tris buffer and the homogenate was centrifuged at 39,000 × g for 10 min at 4°C. The pellet was washed once using the same buffer then suspended in ice-cold 10 mm Tris HCl. Equal aliquots of cell membrane were incubated with ^{125}I-[Tyr-11]SRIF-14 (0.05 nm; 750,000 cpm/ml; 2000 Ci/mmole) and peptide at a final concentration of 10^{-11} to 10^{-6} m in 50 mm HEPES, pH 7.4 containing 1% bovine serum albumin, fraction V, 5 mm $MgCl_2$, Trasylol (200 KIU/ml), bacitracin (0.02 mg/ml) and phenylmethylsulfonyl fluoride (0.02 mg/ml) for 25 min at 30°C. Using a filtration manifold, the mixture was then filtered through a polyethylenimine-washed GF/C filter, and the residue was washed three times with 5 ml ice-cold buffer. The pellet/filter and filtrate/washings were counted in a well- counter to give the fractions of radioactivity bound and free. To assess nonspecific binding, the assay was run in the presence of 200 nm SRIF-14. Analysis of the data gave inhibition constants (Ki) via Hill plots (*22*).

Animal Model

The animal tumor model was essentially that described by Lamberts and Krenning et al. (*23*) and was prepared by A. Bogden, Biomeasure, Inc. CA20948 rat pancreatic tumor brei (0.05 to 0.1 ml) was inoculated into the subcutaneous space of the lateral aspect of the right thighs of 6-wk-old, male Lewis rats (175–225 g). The tumors were allowed to grow to approximately 0.5 to 2 g (2–3 wk) before serial passaging. The tumor-bearing animals used for the in vivo studies were from the fourth to the eleventh passage and carried 0.2 to 2 g (mean 1.2 ± 0.7 g) tumors. The tumors had a stable SSTR density of 80–100 fmole/mg tumor cell protein (assayed using ^{125}I-[Tyr^{11}]SRIF-14) through passages 4 to 9.

For studies of in vivo specificity of radiotracer localization in the tumors, selected animals were given a subcutaneous SSTR-blocking dose of 4 mg/kg octreotide 30 min prior to injection of the radiotracer. This protocol has been shown by Lamberts and Krenning et al. to result in a lowering of ^{111}In-[DTPA]octreotide tumor uptake by 40% (*23*).

In Vivo Tumor Uptake and Kinetics Studies

In all studies, CA20948 tumor-bearing Lewis rats were restrained and injected via the dorsal tail vein with radiolabeled peptides in a 0.2–0.4-ml volume containing 0.20 mCi (7.4 Mbq) of ^{99m}Tc-labeled P587 or P829 (2–8 μg) peptide and/or 0.10 mCi (3.7 MBq) of ^{111}In-[DTPA]octreotide (0.3–1 μg). For imaging studies, the animals were sedated with a mixture of ketamine and xylazine and whole-body images were obtained using a gamma camera (Technicare, Omega 500) fitted with a high-resolution collimator. Images were acquired for 5 min and the data were stored in 128 × 128-image matrix on a Summit image computer system.

Twenty-four Hour Pharmacokinetic Study. Fifteen tumor-bearing rats were injected with ^{99m}Tc-P587 and three animals each were sacrificed at 0.5, 1.5, 3, 6 and 24 hr. Selected tissue samples were excised, weighed and, along with an aliquot of the injected dose, were counted in a gamma well-counter set to count in the ^{99m}Tc window. The results were expressed as percent injected dose per gram (% ID/g) of tissue.

Dual-Tracer Comparison of Technetium-99m-P587 and Indium-111-[DTPA]Octreotide and In Vivo Receptor Specificity Study. Nine of 22 CA20948 tumor-bearing rats were injected subcutaneously with an SSTR-blocking dose of 4 mg/kg octreotide 30 min prior to injection of the radiotracers. All animals were injected with

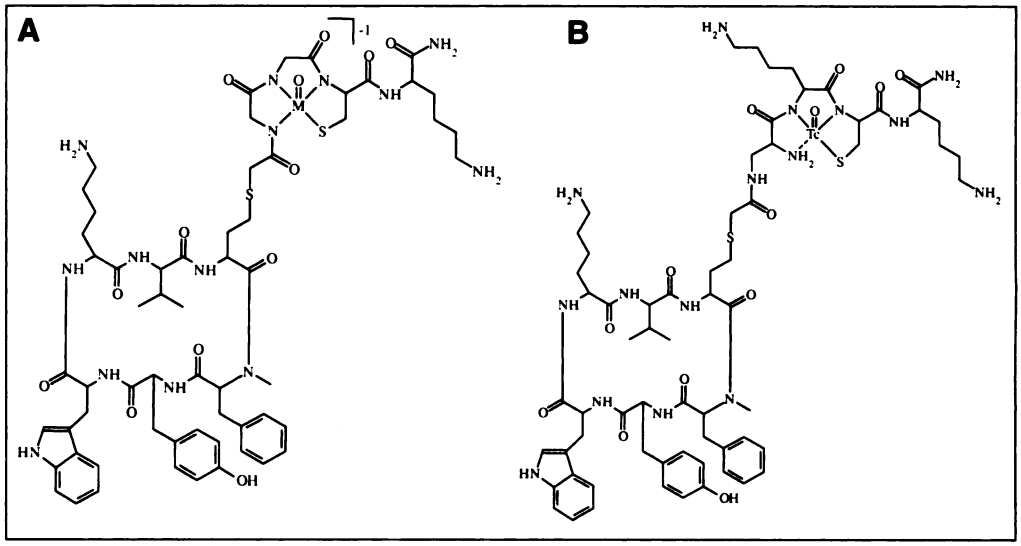

FIGURE 1. Proposed oxotechnetium/oxorhenium complexes of (A) P587 and (B) P829.

^{99m}Tc-P587 followed approximately 1 min later by ^{111}In-[DTPA]octreotide. At 30, 60 and 90 min, three SSTR-blocked and three nonblocked animals were killed by cervical dislocation and selected necropsy was performed. At 180 min the remaining four nonblocked animals were also killed and necropsied. Harvested tissue samples were weighed and, along with aliquots of both injected doses, were counted in a gamma well-counter set to count in both ^{99m}Tc and ^{111}In windows. Technetium-99m counts were corrected for ^{111}In spillover and 3 days later the samples were recounted for ^{111}In only. At 30, 60, 90 and 180 min selected animals also were imaged.

Dual-Tracer Comparison of Technetium-99m-P829 and Indium-111-[DTPA]Octreotide. The tumor uptake of ^{99m}Tc-P829 was compared with ^{111}In-DTPA-octreotide in a dual-tracer study using an additional five tumor-bearing rats. All animals were injected with ^{99m}Tc-P829 followed approximately 1 min later by ^{111}In-[DTPA]octreotide. The animals were killed at 90 min postinjection and % ID/g of tumor, blood, muscle, pancreas and gastrointestinal tract were determined as before for ^{99m}Tc and ^{111}In tracers.

Saturability Study. To assess the effect of specific activity on tumor uptake, ^{99m}Tc-P587 was studied in six groups of three tumor-bearing rats each. The animals were co-injected with either 0, 25, 100, 300, 600, 1000 or 4000 μg/kg of P587 peptide added to the standard dose of ^{99m}Tc-P587 (0.2 mCi; 7.4 MBq; 4 μg peptide). The animals were sacrificed at 90 min postinjection and % ID/g of tumor, blood, muscle, pancreas and gastrointestinal tract were determined as before. A similar study was performed with ^{99m}Tc-P829.

Biodistribution of Technetium-99m Peptides

The biodistributions of ^{99m}Tc-P587 and ^{99m}Tc-P829 were studied noninvasively in normal rabbits by gamma-camera imaging over a period of 4 hr. For the first 1 hr, serial static images (5 min per frame) were acquired and subsequent images were obtained at 2, 3 and 4 hr.

RESULTS

P587 peptide, P829 peptide, [DTPA]octreotide, [ReO]P587, [ReO]P829 and In-[DTPA]octreotide were all prepared and purified to >90% purity by HPLC analysis. The amino acid sequences of all of the peptides are shown in Table 1. The structures for P587 and P829 are shown in Figure 1. The proposed structures of the oxotechnetium and oxorhenium complexes of P587 and P829 are shown in Figures 1A and B, respectively. Selected analytical data are shown in Table 2.

The ^{99m}Tc-labeling of P587 and P829 were routinely obtained in >90% radiochemical yield and of >90% radiochemical purity by ITLC and HPLC analysis. No HPLC purification was required. Residual pertechnetate was <1% and ^{99m}Tc species immobile on ITLC analysis (^{99m}Tc-microcolloid or reduced, hydrolysed ^{99m}Tc) was <3%. HPLC analysis of ^{99m}Tc-P587 showed two, closely eluting radioactive components as expected for syn- and anti-oxotechnetium complexes of the -Gly-Gly-Cys-chelating sequence. HPLC analysis of ^{99m}Tc-P829 showed a single species. The ^{99m}Tc-labeled peptides were routinely prepared of high specific activity (50 mCi (2 GBq)/mg peptide; 60 Ci (2.2 TBq)/mmole peptide) but even higher specific activity (1000 Ci (37 TBq)/mmole P587) was readily achievable without significant loss of radiochemical yield. As discussed below, the effective specific activity was likely even higher.

[ReO]P587 was isolated as a mixture of two closely eluting components by HPLC analysis, presumed to be the expected two isomeric complexes. FABMS analysis showed only MH^+ peaks at m/z = 1458 corresponding to the expected mass (P587 − 4H + ReO + H^+) and 1258 corresponding to P587 itself. [ReO]P829 was isolated as a single peak by HPLC analysis. The HPLC analysis of oxorhenium complexes and ^{99m}Tc-labeled peptides showed that the corresponding rhenium and technetium complexes had virtually identical retention times (see Table 2). These data support the use of the oxorhenium complex(es) as a nonradioactive surrogate for ^{99m}Tc-peptides.

TABLE 2
Peptide and Peptide-Metal Complex Data

Peptide	Calc. MW	MH±(FABMS)	HPLC R_t (min)	
P587	1257.7	1258	7.2[1]	
[ReO]P587	1455.7	1458		14.6[4]
^{99m}Tc-P587	—	—		14.7, 14.9[4]
P829	1357.7	1358	5.8[3]	
[ReO]P829	1556.9	1558		12.2[1]
^{99m}Tc-P829	—	—		12.3[1]
[DTPA]octreotide	1392.6	1394	7.1[1]	
In-[DTPA]octreotide	1504.4*	1507	9.4[2]	

*Assumed formula: [DTPA]octreotide + In − 3H

HPLC elution conditions: (a) 0 to 100% Solvent B in 10 min (Delta-Pak column); (b) 10 to 60% Solvent B in 10 min (Delta-Pak column); (c) 20–50% Solvent B in 10 min (NovaPak column).

TABLE 3
Inhibition Constants for Peptides and Metal Complexes Iodine-125-[Tyr[11]]SRIF-14/AR42J Rat Pancreatic Cell Membrane Assay

Peptide	Inhibition constant, Ki (nM) Peptide	Metal-peptide complex	Ratio*
P587	2.5	0.15[a]	20
P829	10	0.32[a]	30
DTPA-octreotide	1.6	1.20[b]	1

*Ratio of Ki of peptide to peptide-metal complex (to one significant figure).
a = [ReO]peptide complex; b = In-DTPA-octreotide.

The in vitro inhibition constants obtained for P587 peptide, P829 peptide and their corresponding oxorhenium complexes and [DTPA]octreotide, and In-[DTPA]octreotide are shown in Table 3. It is noted that [ReO]P587 has a Ki approximately 20 times lower than that of P587 peptide and [ReO]P829 has a Ki 30 times lower than that of P829 peptide. This indicates that the rhenium complexes (and by inference, the ^{99m}Tc complexes) have a SSTR binding affinities that are an order of magnitude higher than those of the parent peptides. In-[DTPA]octreotide and [DTPA]octreotide did not share this property.

Kinetics of Tumor Uptake

Tumor and selected tissue uptake data of ^{99m}Tc-P587 over the course of the 24-hr pharmacokinetic study are presented in Table 4. Technetium-99m-P587 showed rapid, high tumor uptake, maximizing to 5.0 %ID/g at 3 hr, and only slow (68% of maximum remaining at 6 hr) clearance of radioactivity from the tumor over 24 hr. Tumor-to-blood ratio increased throughout the study, reflecting the initial tumor retention and subsequent much faster clearance of radiotracer from the blood than from the tumor. Tumor-to-muscle increased to 6 hr then showed insignificant change over 24 hr.

Tumor Uptake of Technetium-99m-P587 and Technetium-99m-P829 and Comparison with Indium-111-[DTPA]Octreotide

Data from the ^{99m}Tc-P587 and ^{111}In-[DTPA]octreotide dual-tracer study are presented in Table 5. The tumor uptake of both radiotracers was high and although not significantly different over the course of the 3-hr study, in general ^{99m}Tc-P587 and ^{99m}Tc-P829 gave higher tumor uptake than did ^{111}In-[DTPA]octreotide. Although ^{111}In-[DTPA]octreotide had faster blood clearance than ^{99m}Tc-P587 resulting in higher tumor-to-blood and tumor-to-muscle ratios for ^{111}In-[DTPA]octreotide, the ratios of 6.3 and 29, respectively, obtained with ^{99m}Tc-P587 at 90 min were more than sufficient to predict good imaging.

Data from the ^{99m}Tc-P829 and ^{111}In-[DTPA]octreotide dual-tracer study are presented in Table 6. Technetium-99m-P829 also showed high tumor uptake which was higher than that of ^{111}In-[DTPA]octreotide. Moreover, the blood clearance of ^{99m}Tc-P829 was faster than that of ^{99m}Tc-P587 resulting in tumor-to-blood and tumor-to-muscle ratios which were essentially equivalent to those of ^{111}In-[DTPA]octreotide.

The studies in SSTR-blocked animals (Table 5) showed that the tumor uptake of ^{99m}Tc-P587 was reduced by 86% (at 90 min) in SSTR-blocked versus nonblocked animals. Indium-111-[DTPA]octreotide tumor uptake was similarly reduced by 94%. Blood levels did not change significantly and therefore tumor-to-blood and tumor-to-muscle ratios were concomitantly reduced in SSTR-blocked animals. Notably pancreatic uptake was substantially diminished in blocked animals, indicating that the pancreatic uptake was also substantially receptor-mediated.

As a negative control, a much weaker affinity peptide P443 ([DTPA]-(D-Phe)-(4-chlorophenylalanyl)-Tyr-(D-Trp)-Lys-Thr-Phe-Thr-(ε-Lys)-Gly-Cys-NH_2) having a Ki of 7.9 nm as the oxorhenium complex, was also studied in CA20948 tumor-bearing rats. At 90 min postinjection, ^{99m}Tc-P443 gave only 0.42 %ID/g in the tumor and tumor-to-blood and tumor-to-muscle ratios of 0.9 and 2.7, respectively.

Tumor Uptake of Technetium-99m-Peptides: Imaging Studies

Representative gamma camera images (anterior view) of CA20948 tumor-bearing rats 90 min after injection with ^{99m}Tc-P587 are presented in Figure 2. The radiotracer uptake in the tumor in the right hind leg of each animal is clearly seen. Also seen are kidneys and bladder and some gastrointestinal tract uptake. The images show the obvious difference in tumor uptake in an SSTR-blocked animal (left image) and a non-SSTR-blocked animal (right image). Gamma camera images of ^{99m}Tc-P829 in two animals are shown in Figure 3. Again the high tumor uptake of the ^{99m}Tc-labeled peptide is clearly seen.

Effect of Specific Activity on the Tumor Uptake of the Technetium-99m-Peptides

The effect of increasing the amount of co-injected P587 peptide on ^{99m}Tc-P587 tumor uptake is shown in Figure 4A. P587 peptide was added to a standard preparation of ^{99m}Tc-P587 (0.2 mCi; 7.4 MBq; 5 μg P587 peptide), effectively lowering the specific activity. Tumor uptake decreased substantially when >100 μg P587 peptide was co-injected. Thus 100 μg co-injected P587 resulted in a 50% decrease in tumor uptake. Pancreatic uptake was also reduced by increasing amounts of co-injected P587 peptide. Similarly, the effect of decreasing specific activity on the uptake of ^{99m}Tc-P829 by tumor and pancreas is shown in Figure 4B. Blood, muscle and gastrointestinal tract uptake did not change significantly in either the ^{99m}Tc-P587 or ^{99m}Tc-P829 study.

Biodistribution of Technetium-99m-P587 versus Technetium-99m-P829

The gamma-camera images showing the relative distribution of ^{99m}Tc-P587 and ^{99m}Tc-P829 in normal rabbits are presented in Figure 5. The images show that ^{99m}Tc-P587 has substantial

TABLE 4
Technetium-99m-P587 Pharmacokinetic Study in CA20948 Tumor-Bearing Rats*

Time (hr)	%ID/g Tumor	Blood	Muscle	Tumor: Blood	Tumor: Muscle	%ID GI Tract
1.5	4.0 ± 0.64	0.67 ± 0.048	0.13 ± 0.0067	6.0 ± 0.80	30 ± 4.5	45 ± 1.9
3	5.0 ± 0.066	0.35 ± 0.013	0.076 ± 0.0070	14 ± 0.49	66 ± 6.7	49 ± 2.6
6	3.4 ± 0.59	0.075 ± 0.013	0.029 ± 0.0045	46 ± 8.7	120 ± 4.6	60 ± 17
24	0.80 ± 0.13	0.011 ± 0.0011	0.0084 ± 0.0023	69 ± 5.0	97 ± 12	31 ± 11

*Three animals per time point; mean ± s.d.

TABLE 5
Technetium-99m-P587 and Indium-111-[DTPA]Octreotide Dual-Tracer Study in CA 20948 Tumor-Bearing Rats Data from SSTR-Blocked and Nonblocked Animals*

Time (min)		%ID/g			Tumor:	Tumor:	%ID	
		Tumor	Blood	Muscle	Blood	Muscle	Pancreas	GI Tract
				^{99m}Tc-P587				
30	n	3.8 ± 0.18	1.4 ± 0.45	0.36 ± 0.055	2.3 ± 0.030	11 ± 1.0	3.0 ± 0.23	22 ± 1.7
	b	0.69 ± 0.050	1.5 ± 0.29	0.27 ± 0.070	0.48 ± 0.060	2.6 ± 0.6	0.66 ± 0.38	16 ± 2.5
60	n	2.7 ± 0.68	0.93 ± 0.23	0.19 ± 0.028	3.0 ± 0.61	15 ± 2.6	3.2 ± 0.50	25 ± 3.8
	b	0.70 ± 0.052	1.2 ± 0.18	0.25 ± 0.053	0.61 ± 0.060	2.9 ± 0.49	0.32 ± 0.004	27 ± 5.3
90	n	4.1 ± 0.56	0.64 ± 0.030	0.14 ± 0.010	6.3 ± 1.0	29 ± 2.9	3.2 ± 0.060	33 ± 0.8
	b	0.57 ± 0.08	0.74 ± 0.10	0.14 ± 0.025	0.77 ± 0.080	4.1 ± 0.45	0.27 ± 0.030	35 ± 2.2
180	n	3.0 ± 0.66	0.27 ± 0.030	0.050 ± 0.008	11 ± 2.6	61 ± 14	3.1 ± 0.49	36 ± 3.3
				^{111}In-[DTPA]octreotide				
30	n	3.1 ± 0.72	0.76 ± 0.13	0.29 ± 0.22	4.3 ± 1.6	16 ± 10	2.1 ± 0.91	5.6 ± 1.3
	b	0.62 ± 0.17	0.92 ± 0.38	0.27 ± 0.11	0.71 ± 0.11	2.5 ± 1.0	0.38 ± 0.15	5.1 ± 1.0
60	n	3.1 ± 0.46	0.27 ± 0.040	0.071 ± 0.004	12 ± 0.13	43 ± 4.0	2.4 ± 0.080	5.5 ± 0.23
	b	0.33 ± 0.030	0.31 ± 0.050	0.010 ± 0.015	1.1 ± 0.080	3.3 ± 0.54	0.18 ± 0.11	3.2 ± 0.66
90	n	2.9 ± 1.8	0.13 ± 0.070	0.045 ± 0.024	22 ± 3	64 ± 4.3	2.1 ± 0.92	6.5 ± 0.53
	b	0.18 ± 0.017	0.10 ± 0.004	0.031 ± 0.004	1.8 ± 0.19	5.9 ± 0.61	0.075 ± 0.02	2.8 ± 0.06
180	n	2.7 ± 0.57	0.024 ± 0.004	0.014 ± 0.006	120 ± 39	240 ± 120	2.6 ± 0.12	7.0 ± 0.39

*n = 3 for each group except four in 180-min group; mean ± s.d.
1 n = nonblocked; b = SSTR-blocked with 4 mg/kg octreotide s.c. 30 min before dosing.

uptake in the gastrointestinal tract compared to ^{99m}Tc-P829 which is cleared mostly by the kidneys. A quantitative analysis of the images at 1 hr indicated that about 40% of ^{99m}Tc-P587 activity was in the gastrointestinal tract, 25% in the urinary bladder and only 6% in the kidneys. In contrast, 30% of ^{99m}Tc-P829 activity was in the kidneys, 20% in the urinary bladder and less than 5% in the gastrointestinal tract.

DISCUSSION

The presence of the disulfide bridge of octreotide means that labeling this molecule with ^{99m}Tc is problematic because the reducing agent (usually stannous ion) used in ^{99m}Tc labeling can reduce (open) the disulfide bond with consequent considerable loss of receptor-binding affinity. Macke et al. (*24*) has recently reported a ^{99m}Tc-labeled PnAO conjugate of octreotide. They reported, however, using essentially the same tumor model as we have used, only 0.38% ID/g in the tumor, a tumor-to-blood ratio <1 and only 30% reduction of tumor uptake in SSTR-blocked versus unblocked animals. In contrast, very high tumor uptake (4–5% ID/g), tumor-to-blood (6–21) and tumor-to-muscle ratios (21–68) were seen with ^{99m}Tc-labeled P587 and P829. In addition, 85% reduction in tumor uptake in SSTR-blocked animals was seen with ^{99m}Tc-P587 and both ^{99m}Tc-P587 and ^{99m}Tc-P829 tumor uptake was 80–90% by co-injection of large amounts of the respective peptides. These differences suggest that the ^{99m}Tc-labeled PnAO conjugate of Macke et al. did not retain high receptor-binding affinity and gave mainly nonspecific tumor uptake in vivo.

Inspection of the literature reveals that in order to maintain high SSTR-binding affinity, the pharmacophore of an SSTR ligand needs to be conformationally constrained (*25*). Expressly in order to avoid the incompatibility of having a disulfide in a molecule that is to be radiolabeled with ^{99m}Tc under reducing conditions, we designed peptide P587 and P829 to hold the pharmacophore, that is the key four SSTR-binding amino acid residues -Tyr-(D-Trp)-Lys-Val-, in a cyclic configuration that was not susceptible to reductive cleavage. The sequence -Gly-Gly-Cys- of P587, which constitutes a triamide-thiol chelator which would be expected to form a kinetically stable oxotechnetium (+5) complex (*26*), was appended to the thiol group of the side-chain of a the noncritical homocysteine residue of the molecule. The result was that P587 peptide had an in vitro inhibition constant (Ki) of 2.5 nm, showing it to be a high-affinity ligand for the SSTR. Similarly, with P829 comprises the same SSTR binding cyclic peptide with the novel monoamine, bisamide, monothiol chelating sequence -(β-Dap)-Lys-Cys- appended to the homocysteine side-chain. The Ki for P829 was 10 nm but the oxorhenium complex had a Ki of 0.32 nm.

In order to assess the SSTR-binding affinity of the ^{99m}Tc complex of P587 and ^{99m}Tc-P829, we chose to use the oxorhenium (+5) complexes of these two peptides as a ^{99m}Tc

TABLE 6
Technetium-99m-P829 and Indium-111-[DTPA]Octreotide Dual-Tracer Study in CA 20948 Tumor-Bearing Rats (90-Minute Data)*

%ID/g			Tumor:	Tumor:	%ID	
Tumor	Blood	Muscle	Blood	Muscle	Pancreas	GI Tract
			^{99m}Tc-P829			
4.8 ± 1.1	0.29 ± 0.14	0.078 ± 0.015	21 ± 11	68 ± 26	2.4 ± 0.34	8.4 ± 0.5
			^{111}In-[DTPA]octreotide			
2.8 ± 0.43	0.10 ± 0.024	0.048 ± 0.044	29 ± 5.9	90 ± 57	1.6 ± 0.10	5.0 ± 0.50

*n = 3 for each group.

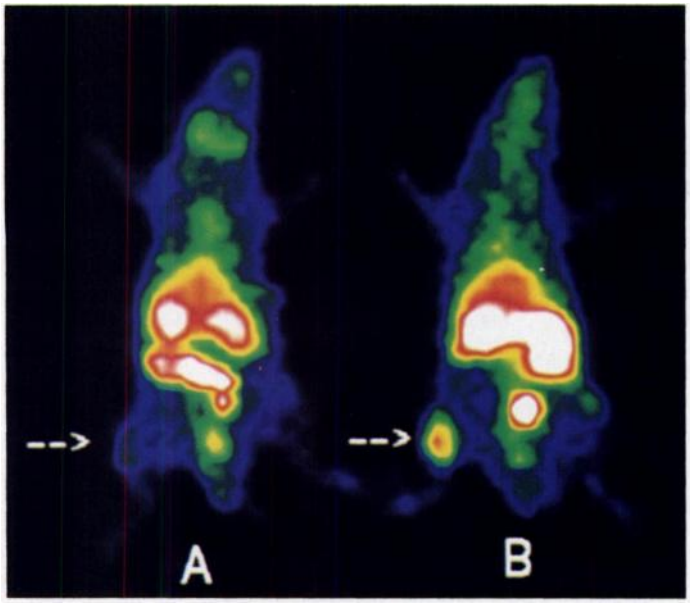

FIGURE 2. Technetium-99m-P587 in a (A) SSTR-blocked (pretreated with octreotide 4 mg/kg) and (B) a nonblocked CA20948 tumor-bearing rat at 90 min postinjection (anterior).

complex surrogate so as to avoid having to use the long-lived isotope ^{99m}Tc in the receptor-binding assay. It has been well-established that technetium and rhenium form configurationally equivalent, albeit not identical, oxometal (+5) complexes of triamide-thiol and bisamide-bisthiol ligands (*27*). As the results show, [ReO]P587 and [ReO]P829 have an even higher SSTR-binding affinity than the parent peptides. The higher affinity of the labeled peptides has important consequences in regard to specific activity. That is that co-injected peptides (P587 or P829) will compete poorly with the ^{99m}Tc-peptide complexes for the somatostatin receptors. Thus a readily achievable specific activity of 1000 Ci/mmole based on total injected P587 may be effectively an order of magnitude higher. In contrast the Kis of [DTPA]octreotide (1.6 nM) and In-[DTPA]octreotide (1.2 nm) which were similar to the previously reported values (*28*), showed that the unlabeled [DTPA]octreotide will compete equally with the ^{111}In-[DTPA]octreotide for the SSTRs.

The in vivo studies in CA20948 tumor-bearing rats showed

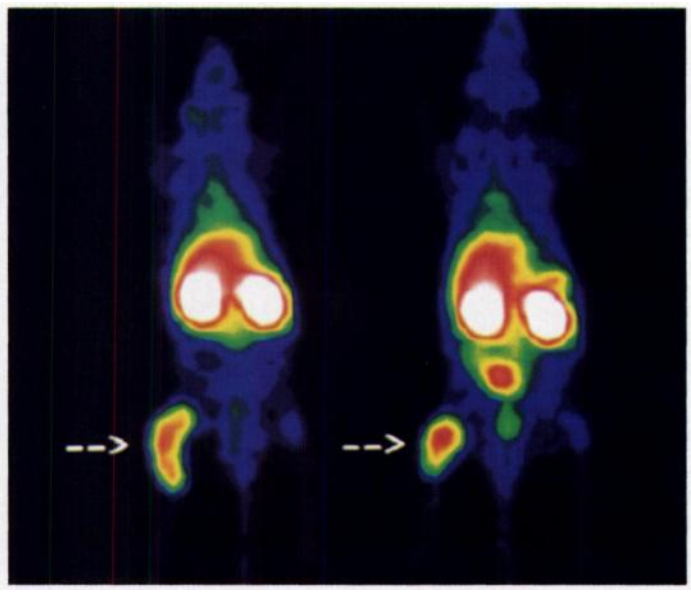

FIGURE 3. Technetium-99m-P829 in CA20948 two tumor-bearing rats (at 90 min postinjection, anterior views).

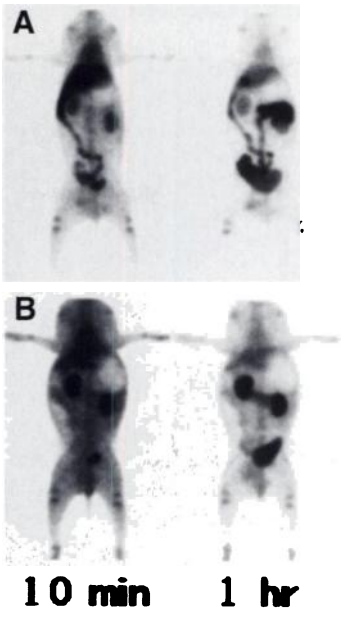

FIGURE 5. Gamma-camera images showing the relative distribution of (A) ^{99m}Tc-P587 and (B) ^{99m}Tc-P829 in normal rabbits at 10 min and 1 hr postinjection.

that the tumor uptake of ^{99m}Tc-P587 and ^{99m}Tc-P829 is at least, and perhaps a little higher than, that of ^{111}In-[DTPA]octreotide, that the tumor uptake of the labeled peptides is specific (blocked by octreotide) and that the tumor uptake of ^{99m}Tc-P587 and ^{99m}Tc-P829 is saturable (diminished by large amounts of co-injected parent peptide).

Regarding the biodistribution of ^{99m}Tc-P587 in normal animals, the biphasic nature of the blood clearance suggested hepatobiliary recycling which was supported by the observed progressive uptake from 15 min to 5 hr in the gastrointestinal tract. Significant reabsorption is probable since less than 6% ID remained in the large intestine at 5 hr and less than 5% ID remained in the entire gastrointestinal tract at 24 hr. Interestingly, the tumor-bearing animals showed greater and more persistent gastrointestinal uptake than did the normal animals. It is noted that as rats do not have gall bladders, biliary secretion is continuous as opposed to being controlled in other species including humans. Thus the gastrointestinal tract uptake seen maximally at from 3 to 6 hr in the rat may occur much later in the human leaving an early window in which the abdomen would be relatively clear of nonspecific radiotracer uptake. The retention of a fraction of the dose in the kidneys indicates proximal tubular reabsorption as has been observed for ^{111}In-[DTPA]octreotide. The primary route of excretion was the renal system as expected for a small peptide and as desirable for a radiopharmaceutical of this type. In comparison, the biodistribution of ^{99m}Tc-P829 showed almost no gastrointestinal uptake, with predominantly renal excretion.

These preclinical results have now been verified in initial clinical studies (*29*) with both agents in which SSTR-expressing tumors were detected as early as 5 min postinjection. Because it has showed both high tumor uptake and low gastrointestinal uptake, ^{99m}Tc-P829 has been selected for clinical studies.

CONCLUSION

Both ^{99m}Tc-P587 and ^{99m}Tc-P829 have been shown to have high SSTR-binding affinity and high, receptor-specific and saturable in vivo tumor uptake, and biodistribution characteristics favorable to early imaging. These observations support the clinical investigation of ^{99m}Tc-P587 and ^{99m}Tc-P829 as radiotracers for the detection of SSTR-expressing tumors and other tissues by gamma scintigraphy.

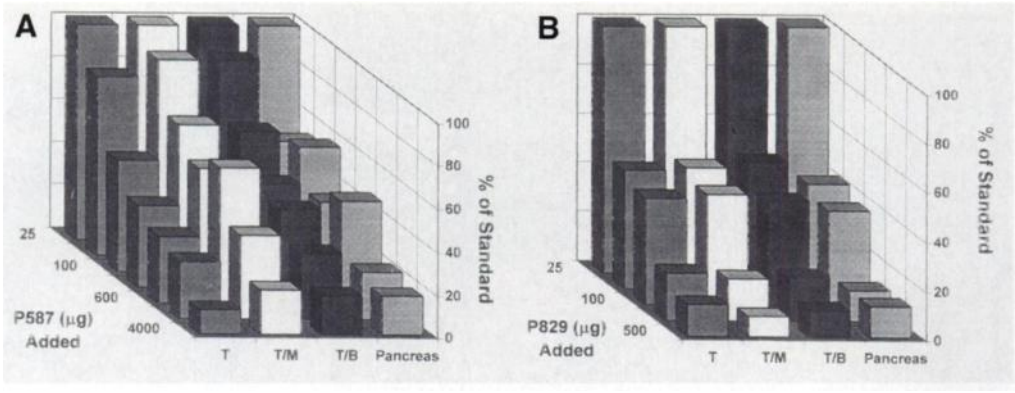

FIGURE 4. Effect of increasing amounts of co-injected cold peptide on the tumor uptake of ^{99m}Tc-peptides in CA20948 tumor-bearing rats. (A) ^{99m}Tc-P587 (top) and (B) ^{99m}Tc-P829. Tumor % ID/g (T), tumor:muscle (T/M), tumor:blood (T/B) and %ID in pancreas were plotted as percent of value observed with a standard (no additional cold peptide) ^{99m}Tc-peptide preparation.

ACKNOWLEDGMENTS

We thank Dr. John E. Taylor, of Biomeasure, Inc., for performing the in vitro assays and Dr. Arthur E. Bogden, also of Biomeasure, Inc., for preparing the animal model.

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