Vapreotide labeled with Tc-99m for imaging tumors: Preparation and preliminary evaluation

M.L. THAKUR[1], H.R. KOLAN[1], S. RIFAT[1], J. LI[1], A. RUX[1], E. JOHN[1], G. HALMOS[2] and A.V. SCHALLY[2]

[1]Thomas Jefferson University, Department of Radiology, Div. of Nuclear Medicine, Philadelphia, PA 19107;
[2]Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical Center, New Orleans, LA 70146 and
Department of Medicine, Tulane Unversity School of Medicine, New Orleans, LA 70146, USA

Abstract. Vapreotide (RC-160), an octapeptide analog of somatostatin, has a high affinity for somatostatin receptor subtypes SSTR2 and SSTR5. Vapreotide binds differently to the tumors of the breast, ovary, exocrine pancreas, prostate and colon, than octreotide another octapeptide analog of somatostatin. Vapreotide was labeled with Tc-99m, a radionuclide highly suitable for scintigraphic imaging. The labeling procedure was simple, produced >70% yields and could be applicable to label other peptides containing a cystine bridge. HPLC analysis showed that the tracer was stable when Tc-99m-RC-160 was challenged with 100 fold molar excess DTPA (diethylenetriaminepentaacetic acid), HSA (human serum albumin) or cysteine and incubated at 37°C for 4 h. HPLC analysis of urine samples obtained from mice that received Tc-99m-RC-160 showed that the preparation was stable *in vivo*. Rat brain cortex membrane receptor displacement assays showed that the Kd values for Tc-99m-RC-160 (71×10^{-9} M) and Tc-99m-octreotide (86×10^{-9} M) (Sandostatin®) were in nM range, and were similar to that for I-125-RC-160 (46×10^{-9} M). High binding affinity of Tc-99m-RC-160 for human breast tumor cells SKBR-3 was also observed. These results suggest that Tc-99m-RC-160 is worthy of evaluation as an agent for scintigraphic imaging of tumors rich in somatostatin receptor subtypes SSTR2 and SSTR5.

Introduction

Vapreotide, also known as RC-160, is a synthetic octapeptide analog of the native hormone somatostatin, produced by the hypothalamus and pancreas (1). Somatostatin is functionally

---

Correspondence to: Dr Mathew L. Thakur, Thomas Jefferson University Hospital, 1020 Locust Street, Suite 359, Philadelphia, PA 19107, USA

described as somatostatin release inhibiting factor (SRIF), because it binds to specific receptors and prevents the secretion of growth hormones possibly by decreasing the adenylate cyclase activity (2-4). In recent years, many endocrine tumors have been found to express excessive quantity of SRIF specific receptors and five subtypes of human SRIF receptors, SSTR1-5, have been cloned (5-8). Among them, SSTR1 and SSTR4 share common characteristics which are different from the subtypes SSTR2, SSTR3 and SSTR5. Vapreotide is reported to have selective high affinity for SSTR2 and SSTR5 (9,10).

Because most of the endocrine tumors and their metastases express SSTRs to a much greater extent than the normal tissues (11-14), another octapeptide analog of somatostatin octreotide (Sandostatin) has been labeled with γ-emitting radionuclides, I-123 or In-111 (In-111-DTPA-octreotide) and been successfully used in imaging a variety of human tumors (15-26). Vapreotide was also labeled with In-111 (17) and with I-123 (27). Encouraging results were obtained, and it was noted that this peptide bound to certain tumors differently than octreotide. These tumors include the cancers of the breast, ovary, exocrine pancreas, prostate and colon (28-32). These observations perhaps suggested that these tumors possessed different receptor subtype density than other tumors.

In radioactive compounds designed as imaging agents, a choice of the radionuclide also plays an important role. Both In-111 and I-123 are cyclotron produced radionuclides. They are, therefore, expensive and must be ordered a day or two ahead of a scheduled procedure. These parameters can be inconvenient to practicing physicians. Technetium-99m (t½=6 h, γ=140 keV - 90%), on the other hand, is a generator produced radionuclide, remarkably inexpensive and it is readily available in all nuclear medicine centers.

We have, therefore, labeled RC-160 with Tc-99m and with stable Re. A two-dimensional ¹H-NMR and computer modeling conformational analysis showed that the Re labeled RC-160 maintained the same overall backbone conformation as the parent molecule (33,34). The purpose of this investigation was to examine the receptor specificity of Tc-99m-RC-160 and to study the *in vitro* and *in vivo* stability of the tracer so that the feasibility of evaluating Tc-99m-RC-160 in pre-clinical animal models could be determined.

Materials and methods

Preparation of Tc-99m labeled RC-160. RC-160 was a generous gift from Debiopharm (Lausanne, Switzerland). Ten µg of RC-160, dissolved in 100 µl 0.01 M acetate buffer at pH 4.2 and 1 mg ascorbic acid in 100 µl H_2O adjusted to pH 6.5 with Na-ascorbate was added. This was followed by the addition of a required quantity (2-20 mCi) of Tc-99m-O_4^- reduced with $Na_2S_2O_4$ which was dissolved in 0.01 M acetate buffer pH 7.2. The final concentration of $Na_2S_2O_4$ was 2 µg/ml reaction mixture which was then heated in boiling water for 15 min.

Alternatively, for a convenient day to day preparations, the following procedure was developed. To 10 ml siliconized glass vials were added 2 mg glycine, 2 mg myo-inositol, 2 mg sodium tetraborate decahydrate, each in 50 µl aqueous solutions. This was followed by the addition of 1 mg Na-ascorbate solution in 100 µl water and 1 mg $Na_2S_2O_4$ in 20 µl 0.01 M Na-acetate buffer pH 7.2. The mixture was quickly vortexed and frozen in acetone/dry ice bath. The vials were then lyophilized, filled with oxygen free nitrogen, sealed with a rubber stopper and an aluminum cap and stored at 4°C.

When it was required, 10 µg of RC-160 in 100 µl 0.01 M acetate buffer pH 4.2 and a required quantity of Tc-99m-O_4^- in 0.9% NaCl were added to the vial followed by an additional volume of 0.9% NaCl to render a final volume of 500 µl. The reaction mixture was then heated in a boiling water bath for 15 min.

In order to eliminate any unbound radioactivity, the mixture was passed through Water's (Milford, MA) C-18 Sep Pak cartridge pre-washed with 5 ml 70% ethanol and 5 ml 2-propanol. The procedure was modified according to Bakker *et al* (35). The cartridge was then washed with 5 ml deionized water, 5 ml 0.5 M acetic acid and then with 5 ml 95% ethanol. Three equal volume fractions of 95% ethanol were collected. Care was taken that the cartridge did not dry out during any stage. Radioactivity in each ethanol fraction was measured in a calibrated ionization chamber (Capintec, NJ). First two fractions containing most of the radioactivity were combined, ethanol was evaporated under a gentle stream of nitrogen and residue was taken up in 0.9% NaCl solution containing 2 mg/ml glycine as a carrier.

Quality control. In order to determine the radioactivity associated with the peptide, instant thin layer chromatography (ITLC, Gelman SG, Ann Arbor, MI) was performed using 85% methanol as a solvent. Radioactivity bound to the peptide remained at Rf. 0.0 and the unbound activity migrated at Rf. 1.0.

HPLC analysis was also performed, using a reverse phase µ Bond Pak C-18 column and 0.9% NaCl as a solvent A and 80% methanol in 0.9% NaCl as a gradient solvent B (0.5 ml/min). The gradient was programmed to start at 50% (A or B), reaching 60% B in 5 min and 90% in 35 min. Rainin's (Emeryville, CA) HPLC equipped with a U.V. detector and a NaI (Tl) radioactivity detector was used.

Preparation of Tc-99m labeled Sandostatin (octreotide). Sandostatin was obtained from the institutional hospital pharmacy and was labeled with Tc-99m as described above. The preparations were also analyzed as described previously.

For receptor binding studies a portion of the Sep Pak purified Tc-99m-RC-160 or Tc-99m-Sandostatin were separated using HPLC, as above, and radioactivity was collected.

Preparation of I-125-RC-160. One of the advantages of RC-160 is that, unlike octreotide, RC-160 contains tyrosine which permits a convenient labeling of the compound with I-125. The iodination was carried out as follows.

To a conical glass vial were added 1.4 µg RC-160 in 14 µl 0.05 M acetate buffer pH 6.5 and 20 µl of 0.05 M phosphate buffer pH 7.5. Approximately 1.4 mCi of I-125 (NEN, Billerica, MA) was then added, followed by 1.6 µg chloramine-T in 20 µl 0.05 M phosphate buffer pH 7.5. The vial was closed, the mixture was vortexed and allowed to incubate for 1 min. The reaction was terminated by the addition of 51 µg cysteine (5.1 mg/ml, in 0.05 M phosphate buffer pH 7.5) and 0.5 ml of 5 mM ammonium acetate. The solution was then processed with Sep Pak purification and HPLC separation as described.

In vitro stability. Following Sep Pak purification and ITLC analysis, a 10 µg Tc-99m-RC-160 preparation, was divided into 3 aliquots, which were then challenged with 100 fold molar excess of cysteine, DTPA (diethylenetriamine-pentaacetic acid) or HSA (human serum albumin). The solutions were incubated at 37°C for 4 h and analyzed by HPLC. The areas under the two peaks were computed by the Rainin program. The sum of the areas under the two Tc-99m-RC-160 peaks in the initial preparation was considered as 100%.

In vivo stability. Four normal Balb/c nude mice weighing between 16-18 g were each given, i.v. approximately 100 µCi of Tc-99m-RC-160. Urine was collected for 4 h and combined. The urine was filtered through 0.22 µ filter to eliminate any particulate matter and then analyzed by HPLC. Radioactivity retained on the filter was negligible. HPLC elution profiles were compared to that of the original preparation and analyzed qualitatively.

Receptor binding assay. Preparation of rat brain cortex membrane. Rat brain cortex membrane was chosen as a source of somatostatin receptors (36). Tissues were prepared using a modified method of Raynor and Reisine (37). Three adult Sprague Dawley rats were sacrificed by decapitation, and the brains were quickly removed. The cortex was dissected and immediately placed in ice cold Hank's balanced salt solution (HBSS) pH 7.5. HBSS was supplemented with 50 µl/ml penicillin, 50 µg/ml streptomycin, 100 i.u./ml Ribonuclease complex (Gibco, USA) and with 10,000 Kallikrein inhibitors units/l (KIU/l) aprotinin. The cortex was then thoroughly rinsed twice with cold HBSS, cut into small pieces and minced using two surgical blades in 10 ml fresh HBSS on ice. The fine, uniform cell aggregate suspension was then transferred into two sterile 50 ml Sorvall (DuPont) test tubes and diluted with 40 ml ice cold HBSS. The tubes were then centrifuged at 500 x g for 10 min at 4°C, supernatant was removed and placed on an ice bath. The

pellet was resuspended in 20 ml homogenization buffer (25 mM Tris-buffer pH 7.5) containing 0.3 M sucrose, 0.25 mM phenylmethyl sulfonyl fluoride (PMSF), 1 mM EGTA, and 10,000 KIU/l aprotinin. The pellet was aspirated in a 10 ml syringe several times and another 30 ml homogenization buffer was added. The homogenate was centrifuged as above and the pellet was homogenized in the same way for 3 more times, saving the supernatant after each centrifugation. The combined supernatants were then centrifuged at 48,000 x g for 45 min at 4°C in Beckman RC-5 centrifuge using a Sorvall SS-34 rotor. Supernatant was discarded and the pellet was washed twice with 50 mM Tris buffer (pH 7.5) containing 5 mM $MgCl_2$, 20 mg/l bacitracin, 0.25 M PMSF, 100,000 KIU/l aprotinin and 1000 i.u./ml RNase inhibitor. The final pellet was resuspended in 5 ml of the washing buffer, separated into 50 μl aliquots and frozen immediately on acetone/dry ice bath and stored at -80°C.

Membrane protein concentration was measured spectrophotometrically using BioRad (Cambridge, MA) protein assay. BSA (bovine serum albumin, 1 mg/ml) was used as a standard.

Receptor binding assays. Receptor binding assays were performed in triplicate in borosilicate test tubes, using rat brain cortex membrane equivalent to 40 μg protein in 150 μl Tris buffer (50 mM, pH 7.5) containing 0.2% BSA, 5 mM $MgCl_2$, 20 mg/l bacitracin, 0.25 mM PMSF, 100,000 KIU/l aprotinin and 15 μg/ml glycine. HPLC purified Technetium-99m labeled or I-125 labeled monoiodo RC-160 were added in a volume of 50 μl to each test tube containing the membrane. The final RC-160 concentration ranged between 10^{-12} to 10^{-3} M. Incubation was carried out at 22°C for 90 min with occasional gentle mixing. Incubation was stopped by rapid filtration through Whatman GF/C filters treated with 0.5% polyethyleneimine and 0.1% BSA in 0.9% NaCl. Filters were washed twice with 1 ml 50 mM Tris buffer (pH 7.5) containing 0.2% BSA. Radioactivity associated with the filters and filtrates, combined with the washes, was counted using Packard 5000 series γ-counter and percentage of radioactivity bound to the membrane was calculated. The percentage of radioactivity bound non-specifically to the membrane at 10^{-3} M concentration was subtracted and data were computed using the Munson Ligand binding program (NIH, Bethesda, MD). Non-specific binding of radioactivity to the filters (without membrane) was also examined and was negligible. Receptor binding assays were also performed with Tc-99m-octreotide (Sandostatin) prepared using the procedure similar to that for Tc-99m-RC-160 described above.

In an additional assay for Tc-99m-RC-160, human breast tumor cells, SKBR-3, grown in tissue cultures were used, instead of rat cortex brain membrane. Each test tube contained an equal number (5×10^6) of cells. The cell concentration was determined using a hemocytometer. The rest of the procedure was unchanged.

Results

Labeled RC-160: Preparation and quality control. The amino acid sequence of RC-160 is shown in Fig. 1. The key
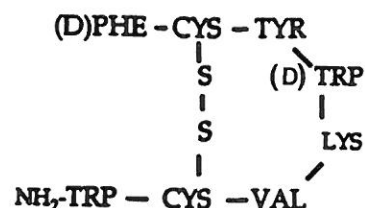

Figure 1. Amino acid sequence of somatostatin octapeptide analog Vapreotide (RC-160).

structural feature of somatostatin and its cyclic peptide analogs such as RC-160 is the type of II' ß-turn located at highly conserved Trp^4-Lys^5 residues (38). Technetium-99m is known to form a strong metal thiolate complex with cysteine sulfur atoms (39). Taking advantage of this technetium chemistry, we have developed a method to label monoclonal antibodies with Tc-99m in which sulfhydryls for Tc-99m chelation were generated by controlled reduction of antibody disulfide bridges with sodium ascorbate (40). Using the same procedure, RC-160 was reacted with sodium ascorbate to reduce the disulfide bonds which provided the binding site for Tc-99m or stable Re. Two dimentional ^{1}H-NMR and computer modeling conformational analysis studies (33) performed with Re-RC-160 complex have shown that Re may have been octahedrally coordinated with four of the RC-160 ligands, Phe^1, Cys^2, Cys^7 and Trp^8. The data also showed that the overall backbone conformation of the parent RC-160 was not altered. In this study, stable Re was chosen because of the similarity of its chemistry with Tc. It is therefore reasonable to assume that Tc-99m formed a similar complex with RC-160.

Following radiolabeling as described, the yields of both Tc-99m and I-125 preparations were very high. Generally more than 70% of the radioactivity was in the first one ml ethanol fraction. The quantity of radioactivity collected in this fraction was dependent on the starting activity the upper limit of which was not examined.

In the HPLC elution curve of I-125-RC-160, there were always two peaks, separating monoiodo and diiodo compounds by five minutes retention time (R.T.). In the monoiodo peak, approximately 66% of the radioactivity was recovered and used.

For Tc-99m-RC-160, generally the major proportion of radioactivity was eluted in one peak. This emerged at approximately 6 min after the peak of unlabeled RC-160 indicating the lipophilic nature of the complex. This separation permitted the collection of Tc-99m-RC-160 (R.T. 21.9 min) in pure form free of unbound RC-160 (RT 11.4 min). In most preparations, however, the radioactivity was eluted in two radioactive peaks, first at R.T. 16.7 min and another at the usual R.T. 21.9 min (Fig. 2). The intensity of this first peak (16.7 min) varied from preparation to preparation, but was generally less than that of the 21.9 min peak. Tc-99m-octreotide was also eluted in two HPLC peaks, the first being eluted earlier (R.T. 10.2) than the first peak of Tc-99m-RC-160. The exact chemical nature of these compounds was not investigated. For *in vitro* and *in vivo* stability studies these peaks, irrespective of their proportions, were not separated from each other.
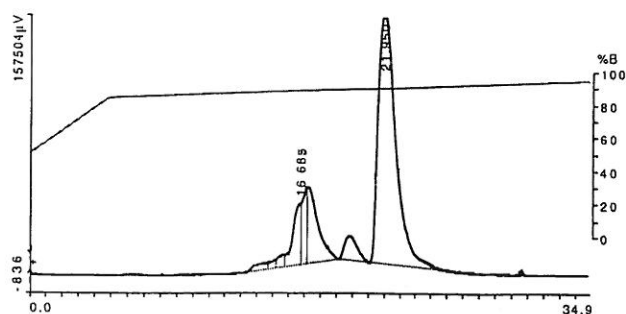
Figure 2. A typical HPLC elution profile of Tc-99m-RC-160. In one peak preparation, the radioactivity elutes at approximately 21 to 22 min RT.
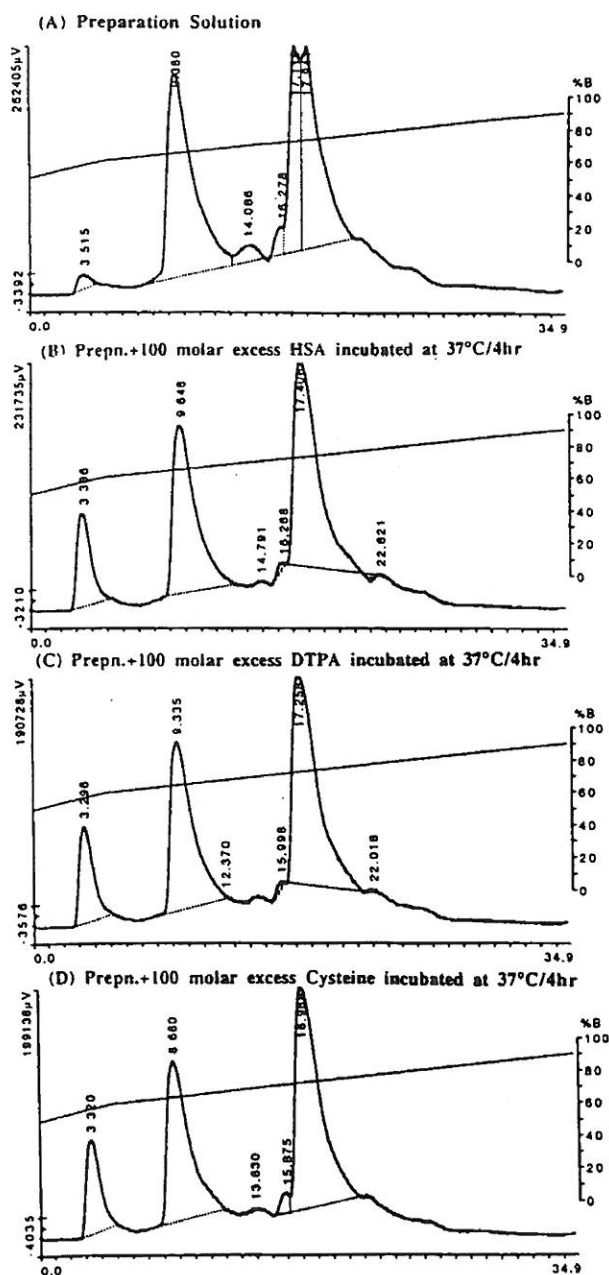

Figure 3. HPLC elution profile of a Tc-99m-RC-160 preparation (A), and of the samples obtained after the preparation was incubated at 37°C for 4 h with 100 fold molar excess of HSA (B), 100 fold molar excess of DTPA (C) and 100 fold molar excess of cysteine (D). The radioactivity peaks at RT 3.2 min represent unbound Tc-99m (A) or Tc-99m bound to the challenging agents (B, C, and D). The radioactivity at 15.7 and 20.8 min (±0.2 min) represent Tc-99m-RC-160. Note that in B, C and D only a small (14.3%, 13.8% and 17.6% respectively) proportion of radioactivity was dissociated from Tc-99m-RC-160.
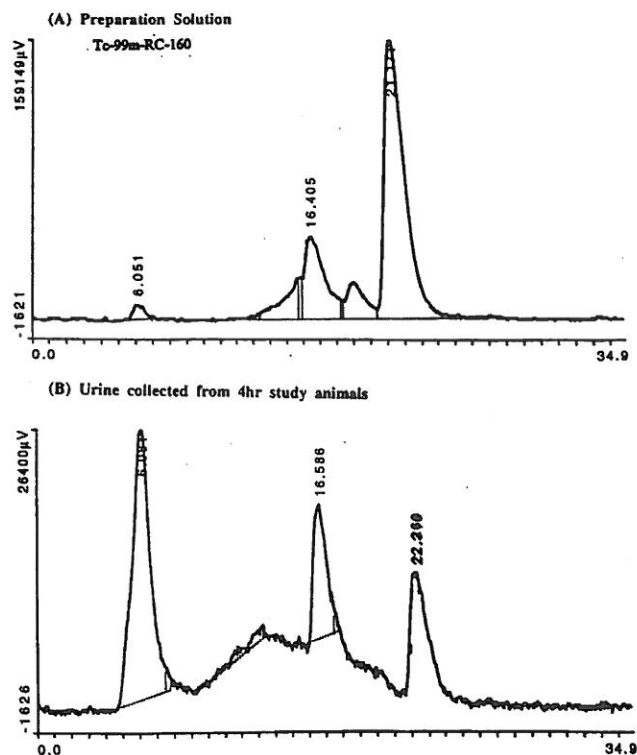

Figure 4. HPLC elution profiles of a Tc-99m-RC-160 preparation (A) and of a sample of urine collected from mice given the preparation 4 h previously (B). Note the similarity of retention times of both radioactivity peaks in the preparations and the urine sample. The third peak in the urine sample at the lower retention time (6.08 min) represents either Tc-99m dissociated from Tc-99m-RC-160, *in vivo* or its metabolite. It has the same retention time as unbound Tc-99m (A) which is longer than that in Fig. 3 because of a longer (20 cm vs 30 cm) column.

In vitro stability. HPLC profiles of Tc-99m-RC-160, incubated for 4 h at 37°C with 100 fold molar excess of DTPA, HSA and cysteine, indicated (Fig. 3) that only approximately 13.8%, 14.3% and 17.6% of radioactivity respectively was dissociated as compared to the percentage in original compound. These challenging agents and their quantities for such stability studies have been in use in our laboratory since 1990 and are considered acceptable (40).

In vivo stability. HPLC elution of urine collected at 4 h from mice given the Tc-99m-RC-160 also indicated (Fig. 4) that a large proportion of the radioactivity excreted in the urine had the same retention times as those of the agent injected.

Receptor specificity. The results of the ligand competition assays as plotted using the NIH computerized curve-fitting program are given in Fig. 5. The non-specific binding at 10^{-3} M was less than 10% of the total binding. Although some data points were scattered, the dissociation constant (Kd) value for Tc-99m-RC-160 (71×10^{-9} M), which was automatically determined by the program, was similar to that of I-125-RC-160 (86×10^{-9} M). Similarly the Kd values for Tc-99m-Sandostatin (95×10^{-9} M) were also in the same nM range. In addition to the rat brain cortex membrane, Tc-99m-RC-160 also showed a high binding affinity (Kd=46×10^{-9} M) for the human breast tumor cell line SKBR-3.
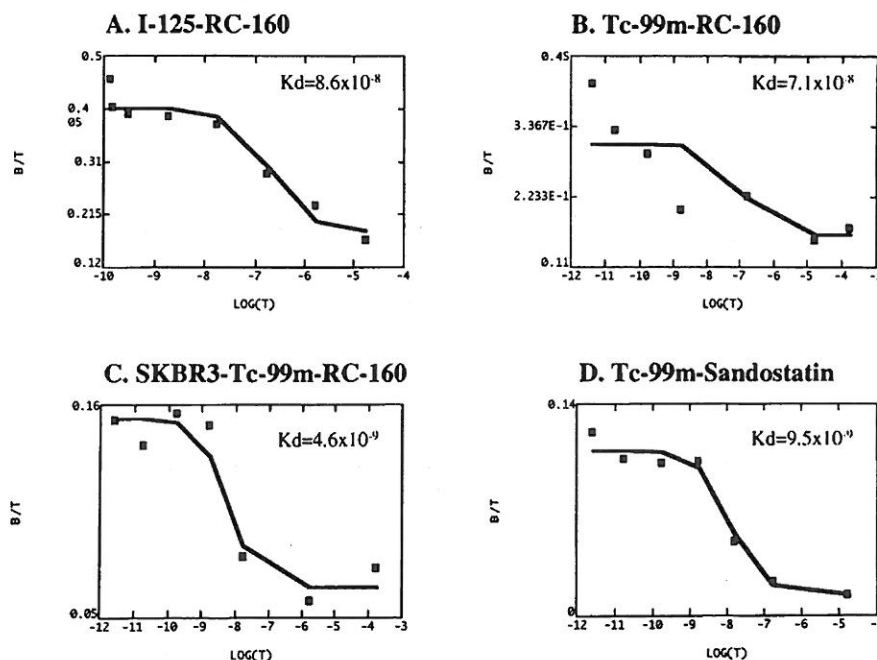
Figure 5. A composite of 4 ligand binding curves. A, B and D, are binding to rat brain cortex membrane, of I-125-RC-160, Tc-99m-RC-160 and Tc-99m-Sandostatin respectively. Curve C, represents displacement binding of Tc-99m-RC-160 to human breast tumor cells SKBR-3. X axis is the corresponding peptide concentration in mol/l. The Kd values for each agent are given in the corresponding box.

Discussion

There are compelling reasons for investigating receptor specific radiolabeled biomolecules for early diagnosis of malignant tumors. Advances in molecular biology have led investigators to identify a large number of glycoprotein on tumor cell surfaces, and a variety of biomolecules, small or large in size, that will selectively interact with these receptors. Radiolabeled monoclonal antibodies specific for many tumor cell surface glycoproteins have been evaluated in experimental animals and patients. However, none so far has made a significant impact in clinical management of cancer patients. The availability of radiolabeled somatostatin analog In-111-DTPA-octreotide has generated new impetus in nuclear medicine and contributed to the era of molecular nuclear medicine. Such agents may not only localize occult tumors at an early stage and simplify the strategy of patient management but can also contribute significantly to the determination of the effectiveness of radiotherapy or hormonal intervention of tumors (25).

However, there is considerable evidence which suggests that another octapeptide analog of somatostatin, Vapreotide or RC-160, binds to certain types of tumors differently from octreotide. Breeman, et al (17) therefore labeled RC-160 with In-111 using DTPA as a bifunctional chelating agent [In-111-DTPA-RC-160] or with I-123 (Tyr³) and evaluated them as agents for imaging experimental tumors in the rat (17,27). Generally with both agents the tumor uptake as well as the uptake of radioactivity in most normal organs were higher than the corresponding preparations of octreotide. These data, complementary to the equally encouraging observations of others (28-32), prompted us to label RC-160 with Tc-99m and evaluate suitability of Tc-99m-RC-160 for in vivo imaging.

The method we have developed to label RC-160 with Tc-99m is simple, reliable and is adaptable to a convenient kit formulation. Although it is based upon the reduction of a disulfide bridge to promote Tc-99m-thiolate chelate chemistry which is frequently used in the preparation of Tc-99m labeled monoclonal antibodies, it has not been applied to small molecular weight peptides. It may, therefore, be considered contrary to the well-known need for cyclization of small peptide molecules for reducing the rate of their proteolysis in vivo. In order to facilitate the structural conformation of RC-160 following reduction of the cysteine bridge, we labeled the peptide in large quantities with stable Re. The two dimensional ¹H-NMR and computer modeling analysis of this complex suggested that the spatial topography of the side chains essential for somatostatin receptor binding of RC-160 was maintained and that the overall backbone conformation of the molecule was retained. The present study was undertaken only after this evidence was obtained.

It was encouraging to observe that the in vitro stability of the agent was sustained when challenged with HSA, a strong chelating agent DTPA, and more importantly, with cysteine. Complementary results were also provided by urine analysis, which indicated that the agent withstood proteolytic degradation for at least up to 4 h. Although the radioactivity peaked at RT 21.7 min appeared more susceptible to degredation, these data were particularly important since such a lack of rapid degradation may allow the agent to interact with the tumor cells long enough to reach sufficient concentration for imaging tumors in vivo. HPLC analysis of urine collected from animals receiving Tc-99m-octreotide, prepared similarly, also showed that Tc-99m-octreotide was stable in vivo (data not shown).

In addition to the stability of the tracer, the preservation of the biological activity of the agent was also important for the optimal use of the agent *in vivo*. The binding affinity of Tc-99m-RC-160, as evaluated *in vitro* using rat brain cortex membrane rich in somatostatin receptors compared favorably with that of I-125 labeled RC-160 as well as with Tc-99m-octreotide. The receptor specificity of RC-160 was further examined using somatostatin receptor positive human breast cancer cells SKBR-3. The Kd values were similar to those of In-111-DTPA-octreotide, or In-111-DTPA-RC-160, which were also in a nM range (17,36).

It is evident from Figs. 2, 3 or 4 that with Tc-99m-RC-160 prepared by this method, we obtained one or two radioactive peaks on reverse phase HPLC. The elution time varied slightly due either to the length of the HPLC column used or to the slightly variable delay in time at which data collection was initiated after an HPLC injection. We have observed that after adding Tc-99m-O$_4^-$ to a kit vial, we obtained two peaks if we thoroughly mixed the vial content before heating. However, if we did not thoroughly dissolve the content before heating, we obtained a single radioactive peak. We believe that the second peak in the well mixed preparation might have been due to different (2$^+$ or 3$^+$) oxidation state of Tc-99m rather than to the nearly 100% of 4$^+$ oxidation state in the unmixed preparations. Although for practical purposes, the preparations with one or two peaks did not seem to make a difference in stability or biological behavior, (data not shown), it will be interesting to examine the chemical nature of the two peaks and further work toward this goal continues in our laboratory. Apparently, elution of Tc-99m labeled peptides in multiple HPLC peaks is not uncommon (41).

The procedure for labeling RC-160 with Tc-99m described in this communication is simple, convenient and eliminates the need for conjugating to the peptides with chelating agents and facilitated Tc-99m binding (42,43). This method has been shown to label octreotide and another peptide, oxytocin, with amino acid sequence H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH$_2$ cyclized via a cystine bridge in position 1 and 6. The method can, in principle, be used for labeling with Tc-99m, stable Re or the radionuclides of Re, namely Re-186 and Re-188, all peptides that contain a cystine bridge in their structure. The biological activity of these peptides, following such a labeling, must be carefully examined before their intended applications are carried out *in vivo*. Furthermore, the results of this method may be verified by comparing them with those obtained from labeling the peptide with I-125 or with Tc-99m using a suitable bifunctional chelating agent.

Acknowledgments

References

1. Srkalovic G, Cai RZ and Schally AV: Evaluation of receptor for somatostatin in various tumors using different analogs. J Clin Endocrinol Metab 70: 661-669, 1990.
2. Brazeau P, Vale W, Burgus R, *et al*: Hypothalamic polypeptide that inhibits the secretin of immunoreactive pituitary growth hormone. Science 179: 77-79, 1973.
3. Vale W, Brazeau P and Rivier C, *et al*: Somatostatin. Recent Prog Horm Res 31: 365-397, 1975.
4. Yamada Y, Post SR and Wang K, *et al*: Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney. Proc Natl Acad Sci USA 89: 251-255, 1992.
5. Ren-Domiano S, Law SF, Yamada Y, *et al*: Pharmacological properties of two cloned somatostatin receptors. Mol Pharmacol 42: 28-34, 1992.
6. Raynok K, Murphy WA, Coy DH, *et al*: Cloned somatostatin receptors: Identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. Mol Pharmacol 43: 838-844, 1993.
7. Raynor K, O'Carroll AM, Kong H, *et al*: Characterization of cloned somatostatin receptors SSTR4 and SSTR5. Mol Pharmacol 44 : 385-392, 1993.
8. Reubi JC, Laissue J, Waser B, Horisberger U and Schaer JC: Expression of somatostatin receptors in normal, inflamed and neoplastic human gastrointestinal tissues. Ann NY Acad Sci 733: 123-137, 1994.
9. Bruns C, Weckbecker G, Raulf R, Kaupmann K, Schoeffer P, Hoyer D and Lubbert H: Molecular pharmacology of somatostatin receptor subtypes. Ann NY Acad Sci 733: 138-146, 1994.
10. Lamberts SW, van der Lely AJ, Derherder WW and Hofland LJ: Octreotide. N Engl J Med 334: 246-254, 1996.
11. Lamberts SW, Krenning EP and Reubi JC: The role of somatostatin and its analogs in the diagnosis and treatment of tumors. Endocrine Rev 12: 450-482, 1991.
12. Reubi JC, Krenning EP, Lamberts SW and Kvols L: Somatostatin receptors in malignant tissues. J Steroid Biochem Mol Biol 37: 1073-1077, 1990.
13. Eden PA and Taylor JE: Somatostatin receptor subtype gene expression in human and rodent tumors. Life Sci 53: 85-90, 1993.
14. Reubi JC, Schaer JC, Waser B and Mengod G: Expression and localization of somatostatin receptor SSTR1, SSTR2 and SSTR3 messenger RNAs in primary human tumors using *in situ* hybridization. Cancer Res 54: 3455-3459, 1994.
15. Bakker WH, Krenning EP, Breeman WA, Kooij PP, Reubi JC, Koper JW, De Jong M, Lameris JS, Visser TJ and Lamberts SW: *In vivo* use of a radioiodinated somatostatin analogue: Dynamics, metabolism, and binding to somatostatin receptor-positive tumors in man. J Nucl Med 32: 1184-1189, 1991.
16. Becker W, Marienhagen J, Scheubel R, Saptogino A, Bakker WH, Breeman WA and Wolf F: Octreotide scintigraphy localizes somatostatin receptor-positive islet cell carcinoma. Eur J Nucl Med 18: 924-927, 1991.
17. Breeman WA, Hofland LJ, ven der Pluijm M, van Koetsveld PM, DeJong M, Setyono-Han B, Bakker WH, Kwekkeboom DJ, Visser TJ and Lamberts SW: Eur. A new radiolabled somatostatin analogue [111-In-DTPA-D-Phe1] RC-160: Preparation, biological activity, receptor scintigraphy in rats and comparison with [111-In-DTPA-D-Phe1] octreotide. J Nucl Med 21: 328-335, 1994.
18. Scherubl H, Bader M, Fett U, Hamm B, Schmidgt-Gayk H, Koppenhagen K, Dop FJ, Riecken EO and Wiedenmann B: Somatostatin-receptor imaging of neuroendocrine gastroentero-pancreatic tumors. Gastroenterology 105: 1705-1709, 1993.
19. Hammon PJ, Arka A, Peters AM, Bloom SR and Gilbey SG: Localization of metastatic gastrenteropancreatic tumors by somatostatin receptor scintigraphy with [111-In-DTPA-D-Phe1] octreotide. Q J Med 87: 83-88, 1994.
20. Nocaudie-Calzada M, Huglo D, Deveaux M, Carnaille B, Proye C and Marchandise X: Iodine-123-Tyr-3-octreotide uptake in pancreatic endocrine tumors and in carcinoids in relation to hormonal inhibition by octreotide. J Nucl Med 35: 57-62, 1994.
21. Van Eyck CH, Bruining HA, Reubi JC, Bakker WH, Oei HY, Krenning EP and Lamberts SW: Use of isotope-labeled somatostatin analogs for visualization of islet cell tumors. World J Surgery 17: 444-447, 1993.
22. Bruns C, Stolz B, Albert R, Marbach P and Pless J: Octreoscan-111 for imaging of a somatostatin receptor-positive islet cell tumor in rat. Horm Metab Res Suppl 27: 5-11, 1993.
23. Joseph K, Stapp J, Reinecke J, Skamel HJ, Hoffken H, Neuhaus C, Lenze H, Trautmann ME and Arnold R: Receptor scintigraphy with 111In-pentetreotide for endocrine gastroenteropancreatic tumors. Horm Metab Res (Suppl) 27: 28-35, 1993.

24. Klapdor R and Montz R: Radioimmunodiagnosis of pancreatic cancer disease. Int J Pancreatol 9: 99-111, 1991.

25. Krenning EP, Kwekkeboom DJ, Bakker WH, Breeman WA, Kooij PP, Oei HY, van Hagen M, Postema PT, DeJong M and Reubi JC: Somatostatin-receptor scintigraphy with [111-In-DTPA-D-Phe1] and [123-I-Tyr3] octreotide: The Rotterdam experience with more than 1000 patients. Eur J Nucl Med 20: 716-773, 1993.

26. Weckbecker G, Raulf F, Stolz B and Bruns C: Somatostatin analogs for diagnosis and treatment of cancer. Pharmacol Ther 60: 245-264, 1993.

27. Breeman WA, Hofland LJ, Bakker WH, van der Pluijm M, van Koetsveld PM, DeJong M, Setyono-Han B, Kwekkeboom DJ, Visser TJ and Lamberts SW: Radioiodinated somatostatin analogue RC-160: Preparation, biological activity, in vivo application in rats and comparison with [123-Tyr3] octreotide. Eur J Nucl Med 20: 1089-1094, 1993.

28. Pinski J, Halmos G and Schally AV: Somatostatin analog RC-160 and bombesin/gastrin releasing peptide antagonist RC-3095 inhibit the growth of androgen-independent DU-145 human prostate cancer line in nude mice. Cancer Lett 71: 189-196, 1993.

29. Radulovic SS, Milovanovic S, Cai RZ and Schally AV: The binding of bombesin and somatostatin and their analogs to human colon cancers. Proc Soc Exp Biol Med 200: 394-401, 1992.

30. Szepeshazi K, Milovanovic S, Lapis K, Groot K and Schally AV: Growth inhibition of estrogen independent MXT mouse mammary carcinoma in mice treated with an agonist or antagonist of LH-RH, an analog of somatostatin, or a combination. Breast Cancer Res Treat 21: 181-192, 1992.

31. Szende B, Srkalovic G, Schally AV, Lapis K and Groot K: Inhibitory effects of analogs of leuteinizing homrone-releasing hormone and somatostatin on pancreatic cancers in hamsters. Eveans that accompany tumor regression. Cancer 65: 2279-2290, 1990.

32. Lamberts SW, Hofland LJ, van Koetsveld PM, Reubi JC, Bruining A, Bakker WH and Krenning EP: Parallel in vivo and in vitro detection of functional somatostatin receptors in human endocrine pancreatic tumors: Consequences with regard to diagnosis, localization and therapy. J Clin Endocrinol Metab 71: 566-574, 1990.

33. Varnum JM, Thakur ML, Schally AV, Jansen S and Mayo KH: Rhenium-labeled somatostatin analog RC-160: ¹H-NMR and computer modeling coformational analysis. J Biol Chem 269: 12583-12588, 1994.

34. Thakur ML, Halmos G, Jan J, John EK, Li J, Mayo K, Rifat S, Rux A, Varnum JM, Wilder S and Schally AV: Technetium-99m RC-160: A somatostatin analog. J Nucl Med 35: 259P, 1994.

35. Bakker WH, Krenning EP, Breeman WA, Koper JW, Kooij PP, Reubi JC, Klijn JG, Visser TJ, Docter R and Lamberts SW: Receptor scintigraphy with a radioiodinated somatostatin analogue: Radiolabeling, purification, biologic activity and in vivo application in animals. J Nucl Med 31: 1501-1509, 1990.

36. Bakker WH, Albert R, Bruns C, Breeman WA, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B and Koper JW: [111-In-DTPA-D-Phe1] octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: Synthesis, radiolabeling and in vitro validation. Life Sci 49: 1583-1591, 1991.

37. Raynor K and Reisine T: Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. J Pharmacol Exp Ther 251: 510-517, 1989.

38. Veber PF: In peptide synthesis: Structure and function. In: Proceedings of the Seventh American Peptide Symposium. DH Rich and VJ Gross (eds). Pierce Chemical Co., Rockford, IL., pp685-694, 1981.

39. Zamora PO and Rhodes BA: Imidazoles as well as thiolates in proteins bind Tc-99m. Bioconjugate Chemistry 3: 493-498, 1992.

40. Thakur ML and DeFulvio JD: Technetium-99m labeled monoclonal antibodies for immunoscintigraphy: Simplified preparation and evaluation. J Immunol Methods 137: 217-223, 1991.

41. Babich JW, Graham W and Fischman AJ: Tc-99m labeled hydrazinonicotinamide conjugated chemotactic peptides: Formulation studies to control radiolabeled speciation. J Nucl Med 36: 148P, 1995.

42. Thakur ML: Radiolabeled peptides: Now and the future. Nucl Med Commun 16: 724-732, 1995.

43. Vallabhajosula S, Moyer BR, Lister-James J, McBride BJ, Lipszyc H, Lee H, Bastidas D and Dean RT: Pre-clinical evaluation of Technetium-99m labeled somatostatin receptor binding peptides. J Nucl Med 37: 1016-1022, 1996.