Radiochemical Investigations of Gastrin-releasing Peptide Receptor-specific [99mTc(X)(CO)3-Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH2)] in PC-3, Tumor-bearing, Rodent Models: Syntheses, Radiolabeling, and in Vitro/t in Vivo Studies where Dpr = 2,3-Diaminopropionic acid and X = H2O or P(CH2OH)3

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ABSTRACT

Bombesin (BBN), a 14 amino acid peptide, is an analogue of human gastrin-releasing peptide (GRP) that binds to GRP receptors (GRPrs) with high affinity and specificity. The GRPrs are overexpressed on a variety of human cancer cells, including prostate, breast, lung, and pancreatic cancers. The specific aim of this study was to develop 99mTc(I)-radiolabeled BBN analogues that maintain high specificity for the GRPr in vivo. A preselected synthetic sequence via solid phase peptide synthesis was designed to produce 2,3-diaminopropionic acid (Dpr)-BBN conjugates with the following general structure: Dpr-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH2). The new BBN constructs were purified by reversed phase high-performance liquid chromatography. Electrospray mass spectrometry was used to characterize the nonmetallated BBN conjugates. Re(I)-BBN conjugates were prepared by the reaction of [Re(Br)3(CO)3]2− and Dpr-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH2) with gentle heating. Electrospray mass spectrometry was used to determine the molecular constitution of the new Re(I) conjugates. The 99mTc conjugates were prepared at the tracer level by preconjugation, postlabeling approach from the reaction of [99mTc(H2O)3(CO)3]- and corresponding ligand. The 99mTc and Re(I) conjugates behaved similarly under identical reversed phase high-performance liquid chromatography conditions. Results from in vitro and in vivo models demonstrated the ability of these derivatives to specifically target GRPrs on human, prostate, carcinous PC-3 cells.

INTRODUCTION

Because of its wide range availability (99Mo/99mTc generator system), ideal nuclear characteristics [1/2 = 6.04 h, Eγ = 140 keV (89%)], and well-established labeling chemistries, 99mTc continues to be the most versatile radioisotope in nuclear medicinal applications today. In fact, 99mTc accounts for >85% of all diagnostic applications performed in medical facilities each year (1). Aside from the traditional approach [i.e., 99mTc(V) or 188Re(V) labeling via N or S chelating donors] of radiolabeling small molecules and biologically active targets with technetium, a versatile radioisotope in nuclear medicinal applications today. In fact, 99mTc(I)-precursor, [99mTc(H2O)3(CO)3]− can act as a radiosynthon for the successful labeling of bioactive molecules with low-valent 99mTc/188Re has been developed (3–6). The new [99mTc(H2O)3(CO)3]− aquaion has been found to be remarkably stable over a wide range of pH values, presumably because of the low-spin, d5 electronic configuration of Tc(I). Furthermore, the lability of the three water molecules coordinated to the fac-M(CO)5 moiety account for excellent labeling efficiencies with a number of donor groups, including amines, thioethers, phosphines, and thiols (3–6).

The feasibility of using the [99mTc(H2O)3(CO)3]− aquaion as a radiosynthon for the successful labeling of bioactive molecules has been reported (6, 22). By simply functionalizing the NH2 terminus of Neurotensin with histidine or (Nα-histidinyl)acetic acid, Alberto et al. were able to successfully radiolabel Neurotensin, achieving relatively high specific activity radiocomplexes. Furthermore, biological activity of the peptide was maintained (22).

In recent years, our laboratory has focused significant effort toward the successful radiolabeling of new BBN analogues to be used as diagnostic and/or therapeutic radiopharmaceuticals in nuclear medicine (23–29). BBN is a 14 amino acid peptide with very high affinity for the GRPr. GRP function and in vivo distribution have been well established. Furthermore, the GRPr is expressed in the central nervous system and peripheral tissues, such as the pancreas or intestinal tract (30–35). A variety of tumors also expresses the BBN receptor/GRPr, including those of breast, prostate, gastric, colon, pancreatic, and small cell lung cancer (30–35). Therefore, radiolabeled BBN/GRPr analogues hold potential to be used as site-directed diagnostic and/or therapeutic targeting motifs. We herein report a new method of radiolabeling the BBN analogue Dpr-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH2) via the 99mTc(I)-precursor, [99mTc(H2O)3(CO)3]−. The in vitro and in vivo efficacy of targeting the GRPr on human, PC-3 cancer cells is reported.

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MATERIALS AND METHODS

\[^{99m}\text{Tc}\] in the form of \[^{99m}\text{Tc}\text{O}_4^-\] was eluted from a \[^{99m}\text{Tc}\text{O}_4^-\] generator provided by Mallinckrodt Medical, Inc. (St. Louis, MO). SPPS techniques, using standard Fmoc chemistry, were used to make all BBN derivatives. SPPS was carried out using an Applied Biosystems 432A peptide synthesizer. Electrospray mass spectral analyses were performed by Synepep Corp. (Dublin, CA). HPLC analyses of radiolabeled and nonradiolabeled compounds were performed on a Waters 600E system equipped with a JASCO UV 975 tunable absorbance detector, an Eppendorf CH-30 column heater, an in-line EG&G ORTEC NaI solid scintillation detector, and a Hewlett Packard 3395 integrator. HPLC solvents were purchased from Fisher Scientific (Pittsburgh, PA) and used without further purification. \[^{99m}\text{Tc}(\text{H}_2\text{O})_6(\text{CO})_3\] (1) was synthesized in a manner similar to that which is reported in the literature (3, 4). \[^{99m}\text{Tc}(\text{Br})_3(\text{CO})_3\] was synthesized in a manner reported previously and was used without further purification (16). All other chemicals were purchased from Aldrich Chemical Co. (St. Louis, MO) and used without further purification.

SPPS

Peptide synthesis was performed on a Perkin-Elmer–Applied Biosystems Model 432A automated peptide synthesizer using traditional Fmoc chemistry. The reaction of the HBTU-activated carboxyl group on the reactant with the NH\textsubscript{2}-terminal amino group on the growing peptide, anchored via the COOH terminus to the resin, provided for stepwise amino acid addition. Rink amide MBHA resin (25 µmol) and Fmoc-protected amino acids, with appropriate side-chain protections, and Fmoc-Dpr(Fmoc)-OH were used for SPPS of the nonmetallated BBN conjugate. The presynthesized sequence was designed to produce the Dpr-(X)-BBN conjugate with the following general structure: Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\textsubscript{2}), 2 (Fig. 1). The final product was cleaved by a standard procedure using a cocktail containing thioanisole, water, ethanethiol, and trifluoroacetic acid in a ratio of 2:1:1:36 and precipitated into methyl-t-butyl ether. The crude peptide was purified by RP-HPLC. Peak purification of the labeled species was performed by collecting the sample off of the chromatographic system into a solution of 1 mg/ml BSA/0.1 M Na\textsubscript{2}HPO\textsubscript{4}. All additional analyses were carried out using the HPLC-purified product.

Synthesis of \[^{99m}\text{Tc}(\text{H}_2\text{O})(\text{CO})_3\text{Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH}_2\text{)}\], 5

To a peak collected sample of the radiolabeled conjugate 4 was added 100 µg of tris(hydroxymethyl)phosphine [P(CH\textsubscript{2}OH)\textsubscript{3}]\text{] in 100 µl of deionized water. The solution was allowed to incubate at room temperature for 1 h. Quality control of the product was determined by RP-HPLC. Peak purification of the labeled species was performed by collecting the sample off of the chromatographic system into a solution of 1 mg/ml BSA/0.1 M Na\textsubscript{2}HPO\textsubscript{4}. All additional analyses were carried out using the HPLC-purified product.

HPLC Analysis of Conjugates 2–5

HPLC analysis of each new compound was performed using an analytical C-18 reversed phase column (Phenomenex, 50 × 4.6 mm, 5 µm). The mobile phase consisted of a linear gradient system, with solvent A corresponding to 100% water with 0.1% trifluoroacetic acid and solvent B corresponding to 100% acetonitrile with 0.1% trifluoroacetic acid. The mobile phase started with solvent compositions of 95% A:5% B at 20 min. At 20 min, the solvent compositions were changed to 90% A:10% B. Solvent compositions of 80% A:20% B were maintained for a period of 5 min, at which point the solvent compositions were changed to 95% A:5% B for column re-equilibration. The flow rate of the mobile phase was 1.5 ml/min. The chart speed of the integrator was 0.5 cm/min.

In Vitro Cell Binding Affinity Studies

In Vitro Receptor Binding.

In vitro receptor binding assay using \(^{125}\text{I}-\text{Tyr}^4\text{-BBN}\) as the radiolabel. Briefly, \(3 \times 10^5\) PC-3 cells suspended in D-MEM/F-12K media containing 0.01 M MEM and 2% BSA (pH 5.5) were incubated at 37°C for 1 h in the presence of 20,000 cpm \(^{125}\text{I}-\text{Tyr}^4\text{-BBN}\) and increasing concentrations of 3. On completion of the incubation, the reaction medium was aspirated, and the cells were washed four times with media. Cell-associated radioactivity was determined by counting in a Packard Rackstar gamma counting system.

Internalization and Eflux Analysis.

In vitro internalization analysis of 4 was carried out by incubation of \(3 \times 10^5\) PC-3 cells [suspended in D-MEM/F-12K media containing 0.01 M MEM and 2% BSA (pH 5.5)] in the presence of 20,000 cpm of 4 at 37°C for selected time points of 10, 20, 30, 45, 60, 90, and 120 min. On completion of the incubation, the reaction medium was aspirated, and the cells were washed four times with media. Surface-bound radioactivity was removed by washing the cells with 0.2 N acetic acid/0.5 M NaCl (pH 2.5). The percentage of internalized, cell-associated radioactivity as a function of time was determined by counting in a Packard Rackstar gamma counting system. Efflux evaluation was performed after a 40-min internalization period. The cellular medium was washed three times with buffer at room temperature and resuspended for further incubation. Selected sampling at 0-, 20-, 40-, 60-, 90-, 120-, and 150-min postincubation was performed by an initial cold buffer wash of the cells, followed by washing with acetic acid/saline (pH 2.5 at 4°C).

Biodistribution Analyses of 4 and 5 in Normal, CF-1, Mouse Models

The biodistribution studies of 4 and 5 were performed in normal, CF-1 mice. The mice were injected with 5µCi (185kBq) of the complex in 50 µl of isotonic saline via the tail vein. The mice were euthanized, and the tissues and organs were excised from the animals after 1-, 4-, and 24-h p.i. Subsequently, the tissues and organs were weighed and counted in a NaI well counter, and the %ID and %ID/gram of each organ or tissue were calculated. The %ID in whole blood was estimated assuming a whole-blood volume of 6.5% the total body weight.

Biodistribution Analyses of 4 and 5 in PC-3 Tumor-bearing SCID Mice

The biodistribution studies of 4 and 5 were performed in SCID mice bearing human PC-3 tumors. Four- to 5-week-old female ICR SCID outbred mice were obtained from Taconic (Germantown, NY). The mice were housed five animals per cage in sterile microisolator cages in a temperature- and humidity-controlled room with a 12-h light/12-h dark schedule. The animals were fed autoclaved rodent chow (Rawlin Purina Company, St. Louis, MO) and water ad libitum. All animal studies were conducted in accordance with the highest standards of care as outlined in the NIH guide for Care and Use of Laboratory Animals.

Fig. 1. Structure of Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\textsubscript{2})\text{], 2.}
Laboratory Animals and the Policy and Procedures for Animal Research at the Harry S. Truman Memorial Veterans’ Hospital. Animals were anesthetized for injections with isoflurane (Baxter Healthcare Corp., Deerfield, IL) at a rate of 2.5% with 0.4 liter of oxygen through a nonrebreathing anesthesia vaporizer.

Human prostate PC-3 cells were injected on the bilateral s.c. flank with \(10^6\) cells in a suspension of 100 l of normal sterile saline per injection site. PC-3 cells were allowed to grow in vivo 2–3 weeks postinoculation, developing tumors ranging in sizes from 0.02 to 1.3 grams.

The mice were injected with \(5 \times 10^4\) Ci of the \(^{99m}\)Tc conjugates in 100 l of isotonic saline via the tail vein. The mice were euthanized, and tissues and organs were excised from the animals at 1-, 4-, and 24-h p.i. Subsequently, the tissues and organs were weighed and counted in a NaI well counter, and the %ID and %ID/gram of each organ or tissue were calculated. The %ID in whole blood was estimated assuming a whole-blood volume of 6.5% the total body weight. Receptor-blocking studies were carried out by administration of 100 g of commercially available BBN in conjunction with the conjugates. The animals were sacrificed at 1-h p.i. The tissues were removed, weighed, and counted as described previously.

**RESULTS**

The Dpr-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) peptide conjugate, 2 (Fig. 1), was conveniently synthesized by SPPS. The yield of the HPLC-purified conjugate was \(\sim 80\%\). ES-MS analysis of the conjugate was consistent with the calculated molecular weight (calculated, 1286.4; experimental, 1287.8).

The \(^{99m}\)Tc(I)-synthon, 1, was prepared by methods similar to those reported previously (Refs. 3 and 4; Fig. 2). The radiosynthon was produced in high yields (\(\geq 95\%\), confirmed by RP-HPLC) on addition of \(^{99m}\)TcO\(_4^-\) to a pressurized, 10-ml serum vial (1 atm of CO) containing NaBH\(_4\) as the reducing agent. The pH of the reaction mixture during the formation of the \(^{99m}\)Tc-precursor 1 was \(\sim 10\). The radiometallated complex 1 was adjusted to a working pH of \(\sim 7.5\) using 0.1 N HCl.

The new, metallated BBN conjugate, 3, was prepared by the addition of an aqueous solution of [Re(Br\(_3\))(CO\(_3\))]\(_2^-\) to the Dpr-(SSS)-BBN Dpr-Ser-Ser-Glu-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) (714)NH\(_2\) peptide with heating. The conjugate was purified by RP-HPLC. Solvent removal under reduced pressure afforded 3 as a pale white solid. Electrospray mass spectrometry allowed for the determination of the molecular ion of the new nonradioactive Re(I) conjugate (calculated, 1557.8; experimental, 1557.8). No dissociation of the fac-Re(I)(CO\(_3\))-mioity was observed from the Dpr-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) ligand framework, demonstrating the stability of the M(I)-N coordinate bond. The ancillary aquo (H\(_2\)O) ligand was not observed in any of the ES-MS analyses. This observation is consistent with coordinating bidentate ligands to low valent Tc(I)/Re(I) metal centers.\(^4\)

\(^4\) R. Schibli, personal communication.
Aliphatic diamine ligands have been found to have relatively slow reaction rates with the \([^{99m}\text{Tc}](\text{H}_2\text{O})_3(\text{CO})_3\)^− moiety as compared with those bidentate ligand frameworks containing an aromatic amine (7). The \(^{99m}\text{Tc}\)-conjugate of the Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) peptide, on the other hand, was produced in high yield on addition of 1 to a vial containing 100 \(\mu\)g (\(~6 \times 10^{-8}\) mol) of 2 with heating (Fig. 2). The radiochemical yield of the new \(^{99m}\text{Tc}\) conjugate was monitored by RP-HPLC. The HPLC chromatographic profile for the HPLC-purified \(^{99m}\text{Tc}\) conjugate of Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) is shown in Fig. 3. The chromatogram shows a single peak (\(t\_R = 16.5\) min) corresponding to the new radiometallated conjugate. It can be concluded that the \(^{99m}\text{Tc}\)-complex of Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) and nonradioactive Re-complex 3 are chemically similar based on the same respective HPLC retention times. Pertechnetate had a retention time of 3 min under identical HPLC conditions.

Over time, the trans-effect of the carbonyl ligand inherently labilizes the coordinating water molecule from the conjugate. In the presence of isotonic saline or dilute HCl, a mixed chlor-aquo species is observed by HPLC at 3-h postpurification (data not shown). The addition of P(CH\(_2\)OH\(_3\)) to the radioconjugate served to displace either the labile H\(_2\)O or Cl\(^-\) ligands (Fig. 2), stabilizing the metal center while also increasing the hydrophilicity of the injected radiopharmaceutical. The radiolabeled conjugate, \([^{99m}\text{Tc}](\text{P(CH}_2\text{OH})_3)(\text{CO})_3\)Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)), 5, was prepared by the addition of 100 \(\mu\)g of tris(hydroxymethyl)phosphine to an HPLC-purified solution of 4 at room temperature. The HPLC chromatographic profile of the new \(^{99m}\text{Tc}\) conjugate is shown in Fig. 3. The chromatogram displays a single peak with a retention time of 15.7 min. This conjugate is stable in aqueous solution for time periods of \(\geq 24\) h.

The metallated Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) derivative exhibits high affinity binding to PC-3 cells, as demonstrated by competitive displacement assays. The IC\(_{50}\) for the metallated conjugate, \([\text{Re}(\text{H}_2\text{O})_3(\text{CO})_3]\)Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)), was found to be 0.86 \(\pm\) 0.22 nM.

Specific binding of the \([^{99m}\text{Tc}](\text{H}_2\text{O})_3(\text{CO})_3]\)Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) conjugate to GRPs expressed on PC-3 cells was demonstrated after incubation (40 min) of \(3 \times 10^4\) PC-3 cells with high specific activity \(^{99m}\text{Tc}\)-analogue. In the absence of the corresponding nonmetallated analogue, \(~3–6\)% of the \(^{99m}\text{Tc}\) activity was associated with the PC-3 cells. In contrast, if \(10^{-5}\) M the corresponding unlabeled Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\)) conjugate or BBN (1–14) is present during the 30-min incubation, \(<0.5\)% of the \(^{99m}\text{Tc}\) activity is cell associated. Fig. 4 summarizes the results of studies to assess the degree of uptake (internalization) of 4 in PC-3 cells. At 90-min postincubation, the amount of internalized activity is 80% of the total activity administered. Fig. 5 summarizes the results of studies to assess the degree of trapping (efflux) of 4 in PC-3 cells. The total \(^{99m}\text{Tc}\) activity associated with the cells after the 40-min incubation was measured after washing the cells with the pH 7.4 incubation media. After washing these cells with the pH 2.5 buffer to remove surface bound \(^{99m}\text{Tc}\) activity, \(~84\)% remained trapped by the cells (Fig. 5). Results of measurements at 20, 40, 60, 90, 120, and 150 min show that activity remains trapped by the PC-3 cells, with \(~46\)% of the \(^{99m}\text{Tc}\) activity associated with the cells at \(t = 0\) remaining residualized at 150 min. Thus, at 150 min, \(~55\)% of the activity remains residualized when normalized to the 84% trapped in the cells at \(t = 0\). The specific trapping mechanism of \(^{99m}\text{Tc}\) activity within the PC-3 cells is not fully understood. It is very likely that lysosomal proteases degrade the conjugate into peptide fragments. Those fragments to which \(^{99m}\text{Tc}\) remains attached are residualized within the cell, within the perinuclear space of the lysosome (36). Additional work is needed to identify the structures of these radiometallated fragments to elucidate the specific trapping mechanisms involved (29).
Thus, the accumulation of $^{99m}$Tc activity in pancreatic tissue at 4- and 24-h p.i. demonstrates GRPr-mediated endocytosis of the ligand framework (Table 1) and tris-hydroxymethylphosphine (Table 2) onto the metal center improved renal-urinary excretion as compared with BBN analogues investigated previously (29). There is no significant uptake or retention in the stomach, indicating that there is minimal, if any, in vivo dissociation of $^{99m}$Tc from this ligand to produce $^{99m}$TcO$_4$\(^{-}\). Pancreatic tissue expresses the GRPr in high density. Therefore, the accumulation of $^{99m}$Tc activity in pancreatic tissue reflects the ability of these derivatives to target GRPr-expressing cells in vivo. Receptor-mediated uptake of 4 and 5 in normal pancreas was $16.3 \pm 1.3$ and $20.5 \pm 4.12\%$ID/gram, respectively. Kidney retention for the $^{99m}$Tc conjugates 4 and 5 was found to be consistent (i.e., $\sim5\%$ID/gram). Blocking studies in which high levels of cold BBN (1–14) was administered 30 min before the $^{99m}$Tc-labeled ligand (37). Recently, we have reported the design and development of $^{99m}$Tc-labeled conjugates of BBN based on the structure N-S-X-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH$_2$)$_X$ [X = 0-Carbons, $\omega$-NH$_2$(CH$_2$)$_2$COOH, $\omega$-NH$_2$(CH$_2$)$_2$COOH, $\omega$-NH$_2$(CH$_2$)$_2$COOH, $\omega$-NH$_2$(CH$_2$)$_2$COOH] (29). $^{99m}$Tc conjugates of N,S-X-BBN(7–14)NH$_2$ were produced in high yield via the prelabeling, postconjugation and postlabeling, preconjugation approaches using $^{99m}$Tc(V)-glucarate as the synthon (29). The $^{99m}$Tc-N,S conjugates were shown to retain high in vitro and in vivo stability and specifically target GRPr-expressing cells in vitro and in CF-1 animal models. Results reported herein, however, suggest the new conjugates 4 and 5 to be superior to the $^{99m}$Tc-N,S conjugates in the same animal model.

The only accessible organ expressing GRPrs is the pancreas, and therefore, notably high pancreatic uptake is observed versus all other tissues. However, significant washout from normal pancreas is observed at 24-h p.i. for each of the two conjugates. Pancreatic uptake and retention for 5, however, is $\sim5\%$ID/gram even at 24-h p.i. for cancerous cells. Biodistribution studies of 5 in tumor-bearing (PC-3) SCID mice showed average tumor uptakes of $2.68 \pm 1.3\%$, $2.58 \pm 1.41\%$, and $1.38 \pm 1.05\%$ at 1-, 4-, and 24-h p.i., respectively (Table 4).

DISCUSSION

This study describes an exciting new approach toward the radiolabeling of GRPr-specific bioconjugates via a "nontraditional" organometallic approach that has been recently described (22). $^{99m}$Tc conjugates 4 and 5 can be prepared in high yield using the preconjugation, postlabeling approach by the reaction of $[^{99m}\text{Tc}](\text{H}_2\text{O})(\text{CO})_3$) with corresponding ligand (37). Recently, we have reported the design and development of $^{99m}$Tc-labeled conjugates of BBN based on the structure N$_3$S-X-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH$_2$) [X = 0-Carbons, $\omega$-NH$_2$(CH$_2$)$_2$COOH, $\omega$-NH$_2$(CH$_2$)$_2$COOH, $\omega$-NH$_2$(CH$_2$)$_2$COOH, $\omega$-NH$_2$(CH$_2$)$_2$COOH] (29). $^{99m}$Tc conjugates of N$_3$S-X-BBN(7–14)NH$_2$ were produced in high yield via the prelabeling, postconjugation and postlabeling, preconjugation approaches using $^{99m}$Tc(V)-glucarate as the synthon (29). The $^{99m}$Tc-N$_3$S conjugates were shown to retain high in vitro and in vivo stability and specifically target GRPr-expressing cells in vitro and in CF-1 animal models. Results reported herein, however, suggest the new conjugates 4 and 5 to be superior to the $^{99m}$Tc-N$_3$S conjugates in the same animal model.

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reasons not fully understood. Tumor uptake and retention were apparent for each of the new \[^{99mTc}\text{I} \]-Dpr conjugates 4 and 5, confirming the agonistic nature of the conjugates (Tables 3 and 4). However, uptake in normal pancreas \textit{versus} tumor is evident and presumably caused by the ability of the conjugates to effectively target the well-vascularized pancreas and GRPrs thereon as compared with the inoculated tumor tissue. It is important to note that recent studies in our laboratory demonstrated successful control of tumors without significant radiotoxicity to the pancreas when targeted with \[^{177}Lu\]/\[^{90}Y\]-labeled BBN conjugates (38). Furthermore, receptor density can vary greatly from rodent models to humans, potentially eliminating any radiotoxicity in human patients (39). Retention of \[^{99mTc}\text{I} \]-activity, even at 24-h p.i., complements the results discussed herein provide for \[^{99mTc}\text{I} \]-labeled conjugates that retain high in \textit{vivo} and \textit{in vitro} specificity targeting of GRPr-expressing cells. It was shown that the structures of these conjugates could be varied with little or no compromise of agonistic binding to GRPrs. The potential clinical utility of a \[^{99mTc}\text{I} \]NS-Ava-Gln-Trp-Ala-Val-Gly-His-Leu-Met(\text{NH}_3)\text{I} construct, designed and developed in our laboratory, as a cancer-specific imaging agent was recently demonstrated by Van de Weille \textit{et al.} (43, 44) in human patients with either prostate or breast cancer. Their studies showed that the N,S conjugate localizes in tumors with high specificity producing good tumor:normal tissue uptake ratios and high-quality SPECT images (43, 44). Tumor uptake and retention in human prostate (PC-3) cells for the new conjugate \[^{99mTc}\text{(H}_2\text{O})\text{I} \]Dpr-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met(\text{NH}_3)\text{I} , 4, is superior to the \[^{99mTc}\text{I} \]NS conjugate in the same animal model (45). However, the clinical superiority of this compound over \[^{99mTc}\text{I} \]NS-Ava-Gln-Trp-Ala-Val-Gly-His-Leu-Met(\text{NH}_3)\text{I} has yet to be established. These results further show the versatility of manipulating each the tethering moiety and ancillary third ligand, providing an effective strategy for optimizing pharmacokinetics of the radiolabeled BBN conjugates.

REFERENCES


5. Alberto, R., Schibli, R., Egli, L., Schubiger, P. A., Herrmann, W. A., Artus, G., Abram, U., and Kaden, T. A. Metal carbonyl syntheses XXII. Low pressure carbonylation of \[^{181}Mo\text{O}3\]2 and \[^{181}Mo\text{O}4\]2: the molybdenum(I) and rhenium(I) complexes \[^{181}Mo\text{O}3\]2\(\text{H}_2\text{O}\)\(\text{O}2\)\(\text{O}2\), \[^{181}Mo\text{O}4\]2\(\text{H}_2\text{O}\)\(\text{O}2\)\(\text{O}2\), and \[^{181}Mo\text{O}4\]2\(\text{H}_2\text{O}\)\(\text{O}2\)\(\text{O}2\). J. Organomet. Chem., 493: 119–127, 1995.


15. Schibli, R., Kati, K. V., Volkert, W. A., and Barnes, C. L. Novel coordination behavior of \[^{99mTc}\text{I} \]CO\text{I} \enate with 1, 3, 5-triaz-7-phosphoamide (PTA). Systematic investigation on stepwise replacement of the halides by PTA ligand. Phase
Radiochemical Investigations of Gastrin-releasing Peptide Receptor-specific \([^{99m}Tc(X)(CO)_3}\) Dpr-Ser-Ser-Ser-Gln-Trp-Ala-Val-Gly-His-Leu-Met-(NH\(_2\))] in PC-3, Tumor-bearing, Rodent Models: Syntheses, Radiolabeling, and \textit{in Vitro}/\textit{in Vivo} Studies where Dpr = 2,3-Diaminopropionic acid and X = H\(_2\)O or P(CH\(_2\)OH\(_3\))


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