Radiotherapy, Toxicity and Dosimetry of Copper-64-TETA-Octreotide in Tumor-Bearing Rats

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The efficacy of ^{64}Cu [$T_{1/2}$ = 12.7 hr; β^+ (0.655 MeV; 19%); β^- (0.573 MeV; 40%)] as a radioisotope for radiotherapy has been recently established. Here we demonstrate that ^{64}Cu-1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA)-octreotide, a somatostatin receptor ligand, inhibits the growth of CA20948 rat pancreatic tumors in Lewis rats at doses that cause minimal toxicity. **Methods:** Tumor-bearing rats were administered a single 15 mCi (555 MBq) dose, a fractionated dose of 15 mCi given in 2–3 doses over 2–8 days, or control agents of buffer, unlabeled octreotide or ^{64}Cu-labeled TETA. In certain experiments, blood was removed at times from 4–23 days post-treatment, and a complete blood count along with blood chemistry analyses were obtained. **Results:** Tumor-growth inhibition was significantly greater in rats injected with a single 15 mCi dose than in rats injected with control agents ($p < 0.05$). Dose fractionation in two doses, either 1 or 2 days apart, induced significantly increased tumor-growth inhibition compared with rats given a single dose ($p < 0.05$). The only toxicity observed in treated rats was a decrease in the white blood cell count. This drop was more pronounced in rats treated with a single dose compared with those treated with a fractionated dose. Human absorbed doses of ^{64}Cu-TETA-octreotide to normal organs were estimated from biodistribution data in Lewis rats, and these data indicate that radiotherapy with ^{64}Cu-TETA-octreotide in humans would be feasible. **Conclusion:** Copper-64-TETA-octreotide is a promising radiopharmaceutical for targeted radiotherapy of somatostatin receptor-positive tumors.

Key Words: copper-64; targeted radiotherapy; somatostatin analog; dosimetry; receptor binding

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The majority of radiopharmaceuticals that have been evaluated as radiotherapy agents are radiolabeled monoclonal antibodies (MAbs); however, in the past 5 yr there have been articles on radiotherapy with radiolabeled small organic molecules and peptides (*1–4*). Potential advantages of small molecules for radiotherapy include lower myelotoxicity due to rapid blood clearance and better tumor penetration due to their small size and lack of immunological response. The use of radiolabeled ligands that bind to somatostatin receptors as radiotherapy agents is increasing (*5,6*), and preliminary results of clinical radiotherapy trials with ^{111}In-diethylenetriamine pentaacetic acid (DTPA)-octreotide (currently used routinely for the scintigraphic localization of primary and metastatic neuroendocrine tumors bearing somatostatin receptors) have demonstrated evidence of tumor response to treatment (*6,7*). There have also been reports of radiotherapy studies in animal models using ^{90}Y-labeled DTPA and DOTA-octreotide conjugates (*2,8,9*), and ^{188}Re direct-labeled RC-160 (*3*), both of which demonstrated inhibition of tumor growth. The biodiodistribution of ^{161}Tb-DTPA-octreotide, another potential radiotherapeutic agent, has also been reported (*10*).

We have investigated the use of ^{64}Cu ($T_{1/2}$ = 12.7 hr) as a radioisotope for radiotherapy both in vitro (*11,12*) and in vivo (*13*). Copper-64 decays by β^+ (0.655 MeV; 19%) and β^- (0.573 MeV; 40%), which enables PET imaging and radiotherapy (*14*). Copper-64 can be produced by either a reactor (*15*) or medical cyclotron (*16*), and we have found it to be as effective as ^{67}Cu for radioimmunotherapy (RIT) with intact MAb 1A3 in a tumor-bearing hamster model (*13*). We have developed a ^{64}Cu-labeled octreotide analog for both PET imaging and radiotherapy (*17*). Copper-64-1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid-(TETA)-octreotide binds to the somatostatin receptor with an affinity similar to ^{111}In-DTPA-octreotide, has desirable clearance properties (renal clearance with rapid excretion) and is currently being investigated at Washington University in research subjects as a PET imaging agent in patients with somatostatin receptor positive tumors (*18*).

One of the aims of this study was to combine the excellent targeting capability of the somatostatin receptor ligand octreotide with ^{64}Cu, a radionuclide with a half-life that is potentially more suited for small peptides and proteins. In this article, we describe the evaluation of ^{64}Cu-TETA-octreotide as a radiotherapeutic radiopharmaceutical in a tumor-bearing rat model. The therapeutic efficacy of ^{64}Cu-TETA-octreotide has been evaluated when administered in either a single dose or in fractionated doses. Estimated human absorbed doses to normal organs, as well as the absorbed dose to the rat tumor, have been determined.

MATERIALS AND METHODS

Preparation of Copper-64-TETA and Copper-64-TETA-Octreotide

Ammonium acetate (NH_4OAc) was purchased from Fluka Chemical Co. (Ronkonkoma, NY); octreotide was purchased from DePaul Pharmaceutical (Bridgeton, MO); 2-(1H-benzotriazole-1-yl)−1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Novabiochem (San Diego, CA); trifluoroacetic acid (TFA) (>99%, Chicago, IL) was purchased from VWR Scientific Products (St. Louis, MO); and bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO). All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) (in >99% purity). Copper-64 was produced at the Missouri University Research Reactor (*15*) and on a biomedical cyclotron (*16*). Specific activity of ^{64}Cu prepared by both methods ranged from 6000–50,000 Ci/mmol. All solutions were made using distilled deionized water (Milli-Q (Millipore; Bedford, MA); > 18 $M\Omega$ resistivity). Reversed-phase high-performance liquid chromatography (HPLC) was performed on a Waters (Milford, MA) 600E chromatography system with a Waters 991 photodiode array detector and an Ortec Model 661 (EG&G Instruments, Oak Ridge, TN) radioactive detector. All samples were processed using Waters

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Millenium 2010 Chromotography Manager. Radio-thin layer chromatography (TLC) was performed using reversed-phase (C-18) plates (Whatman, Fairfield, NJ) and a BIOSCAN System 200 imaging scanner (Washington, DC). Radioactive samples were counted on a Beckman (Arlington Heights, IL) gamma counter containing a NaI crystal. Electrospray mass-spectrometry was performed on a Vestec 201(Houston, TX) mass spectrometer.

TETA-octreotide was prepared in a procedure modified from that published (*17*). Briefly, octreotide was protected with a *tert*-butoxycarbonyl (Boc) group by reaction with $(Boc)_2O$ in dimethylsulfide (DMSO). TETA•4HCl•$4H_2O$ was neutralized with 4.5 equivalents of aqueous LiOH. The N-terminal amine of the Boc-protected octreotide was conjugated to one of the carboxylic acid moieties on TETA with HBTU in DMSO using diisopropylethylamine and hydroxybenzotriazole as catalysts. The reactions were monitored on an Alltech mixed mode reversed-phase C-18-cation (Deerfield, IL) HPLC column (250 mm × 4.6 mm) and the major product (>90%) was collected by semipreparative reverse-phase C18-cation-HPLC on an Alltech mixed mode column (150 mm × 10 mm). For analytical reverse-phase HPLC, the mobile phase was 90:10 H_2O (0.1% TFA):acetonitrile (ACN) (0.1% TFA) (solvent A) and 90:10 ACN (0.1% TFA):H_2O (0.1% TFA) (solvent B). The gradient consisted of 10% B to 60% B in 40 min (1.0 ml/min flow rate). For preparative, HPLC isopropanol (0.1% TFA) was used as a component of the mobile phase in place of ACN (0.1% TFA). TETA-octreotide was produced in ~500 μg batches and aliquoted in 1–50 μg amounts for receptor binding, biodistribution and radiotherapy experiments.

Copper-64 was labeled to TETA-octreotide as described previously (*17*). Briefly, ^{64}Cu-acetate (5–150 mCi) was labeled to 1–50 μg TETA-D-Phe^1-octreotide in 0.1 *M* ammonium acetate buffer, pH 5.5. One milligram of gentisic acid per milliliter was added to the radiolabeled conjugates to reduce the effects of radiolysis. Radiochemical purity was determined by reversed-phase HPLC. Preparations were purified by a C-18 SepPak® (Waters) to remove uncomplexed ^{64}Cu-acetate. Radiochemical purity was assessed by radio-TLC using reversed-phase C-18 plates developed in 70:30 methanol:5% NH_4OAc.

Copper-64-TETA was prepared as follows: ^{64}Cu-acetate (76 mCi) (0.1 *M* NH_4OAc, pH 5.5) was incubated with 100 μg of TETA (0.1 *M* NH_4OAc, pH 5.5) for 30 min at room temperature. Purity was assessed by radio-TLC using silica gel coated plates developed in 1:1 10% NH_4OAc:methanol.

Animal Model

All animal experiments were performed in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee. Male Lewis rats were purchased from Charles River Laboratories (Boston, MA). The somatostatin receptor-positive rat pancreatic tumor CA20948 was obtained from the Tumor Bank at Biomeasure, Inc. (Hopkinton, MA). Lewis rats were implanted with CA20948 rat pancreatic tumors (*19*) in the nape of the neck from the original frozen cells obtained from Biomeasure, Inc., and then tumors were serially passaged from animal to animal. After implantation, the CA20948 tumor goes through a lag phase with little or no observable tumor growth (about 7 days), followed by rapid growth (doubling time 12–24 hr).

Receptor Binding Assay

CA20948 tumors were harvested from euthanized host rats when tumors reached a palpable size or larger (approximate weight between 0.5–2 g). CA20948 membranes were prepared as described by Raynor and Reisine (*20*). The final membrane pellet was diluted to a working concentration of 1200–1300 μg/ml and kept on ice before use.

Copper-64-TETA-octreotide was prepared as described above, at a specific activity of 1800–2100 Ci/mmol. The radiotracer was diluted in receptor buffer [50 m*M* Tris-HCl, pH 7.4; 5.0 m*M* $MgCl_2•6H_2O$; 0.5 μg/ml aprotinin; 200 μg/ml bacitracin; 10 μg/ml leupeptin; 10 μg/ml pepstatin; 0.5 m*M* PMSF (a cholinesterase inhibitor)] to a specific concentration that would give a final concentration of 0.05 n*M* per sample (approximately 30,000–60,000 cpm). Experiments were conducted using the Millipore MultiScreen system (Bedford, MA). Aliquots of 75–150 μg of membrane protein were incubated in triplicate along with 0.05 n*M* radioligand and 13 doses of the competitor ligand (unlabeled copper-TETA-octreotide), covering a concentration range from 0–100 n*M*. Samples were mixed gently and allowed to incubate for 2 hr at room temperature. After incubation, supernatants were aspirated through the filters using a vacuum manifold. Filters were washed twice with 250 μl of ice-cold binding buffer and then air dried. A filter-punch apparatus (Millipore, Inc.) was used to extricate the filters from the plate and dispense them into tubes for counting. The resulting filter samples, along with samples representing total counts added, were counted for radioactivity.

For data analysis, the GraFit program (Erithacus Software, United Kingdom) was used to generate a four-parameter logistic, which allowed determination of IC_{50} values (the amount of cold ligand required to displace 50% of the tracer ligand) and production of a displacement plot. The program LIGAND (*21*) was used to generate binding constants (K_D) and receptor density (B_{max}). LIGAND generates a coefficient of variance (CV), which is an error measurement indicative of sample deviation from the curve fitting analysis.

Single Dose Radiotherapy Experiments

Male Lewis rats were injected with 5 × 10^6 CA20948 rat pancreatic tumor cells 7–9 days before treatment with ^{64}Cu-TETA-octreotide or control agents. Rats were divided into treated or control groups with comparable distribution of tumors sizes among groups. In single dose radiotherapy experiments, rats were injected with one dose of either 0.1% BSA in 0.1 *M* NH_4OAc (vehicle), 5 μg of octreotide in vehicle, 15 mCi of ^{64}Cu-TETA or 15 mCi of ^{64}Cu-TETA-octreotide (n = 5 for each group). At the 15 mCi single dose, experiments were performed in both small (3 ± 2 mm^3; 1–6 mm^3) and large (196 ± 112 mm^3; 103–340 mm^3) tumors (n = 4 for large tumors). The tumor volume was measured every 1–3 days (using calipers), and rats were sacrificed by administration of halothane when the tumors reached a volume of ~10 cm^3 or the tumor became ulcerated. Tumor measurements of very small tumors (<4 mm^3) were estimated from caliper measurements, taking into account the thickness of the skin (about 2 mm). The CA20948 tumors were highly vascularized and, as the tumors grew to a large size (>3000 mm^3), there were often fluid-filled pockets and necrotic regions, which frequently lead to ulceration.

Dose Fractionation Experiments

In four separate dose fractionation experiments, tumor-bearing rats were injected with either 2 or 3 doses of ^{64}Cu-TETA-octreotide (14–15 mCi total), 0.1% BSA in 0.1 *M* NH_4OAc (vehicle) or 1.5–2.5 μg of octreotide in vehicle (n = 5 for each group). In dose fractionation experiments, tumor-bearing rats were injected with either 9 + 5 mCi on days 1 and 3 (average tumor size = 21 ± 10 mm^3; range = 14–34 mm^3), 7.5 + 7.5 mCi on days 1 and 2 (average tumor size = 19 ± 22 mm^3; range = 2.1–27 mm^3), 10 + 5 mCi on days 1 and 1.5 (average tumor size = 2.7 ± 2.5 mm^3; range = 0.5–6.3 mm^3) and 5 + 4 + 5 mCi on days 1, 3 and 8 (average tumor size = 22 ± 24 mm^3; range = 4.2–65 mm^3). The method of administration of vehicle and octreotide controls was similar to that of ^{64}Cu-TETA-octreotide. The dose fractionation

therapy experiments were performed as described for the single dose radiotherapy experiments.

Statistical Analysis

The SAS statistical package (SAS Institute, Cary, NC) was used to analyze data from single dose and dose fractionation radiotherapy experiments compared with control rats injected with vehicle only. Because there were two criteria for sacrifice of the tumor-bearing rats (tumor size of > 10,000 mm^3 or tumor ulceration), we evaluated the time for the tumor size to reach 3000 mm^3. The data were analyzed to determine statistical significance using two methods: (a) standard analysis of variance; and (b) least difference test. The data from rats treated with control agents were compared with data from rats treated with either a single 15 mCi administration of ^{64}Cu-TETA-octreotide or dose fractionation (on Days 1 and 2 or Days 1 and 3). The data from experiments where rats were treated with a single dose were also compared with data from rats who were treated with the fractionated doses.

Toxicity

Preliminary toxicity data were obtained from one group of tumor-bearing rats that received a single 15 mCi treatment (n = 4) and for the group of rats that received a fractionated dose (7.5 + 7.5 mCi 24 hr apart) (n = 5). These results were compared with rats treated with control agents (either buffer or unlabeled octreotide). In each of these groups, the rats were weighed and blood was removed by cardiac puncture from anesthetized rats four times during the survival period post-treatment. The blood was analyzed for hematological values, as well as liver and kidney enzyme levels, at the Diagnostic Services Laboratory in the Department of Comparative Medicine at Washington University School of Medicine. The hematology analysis included white blood cell (WBC), red blood cell, platelet counts, measurement of hemoglobin and hematocrit and differential WBCs. Liver and kidney enzymes analyzed included blood urea nitrogen (BUN), creatinine, total protein, albumin, alkaline phosphatase, alanine amino transferase, aspartate amino transferase and total bilirubin.

Dosimetry

Biodistribution experiments of injected radiolabeled octreotide conjugates were performed in male Lewis rats (220–260 g) carrying CA20948 tumors. The tumor-bearing Lewis rats were anesthetized with Metofane®, injected intravenously with 1.2 MBq (30 μCi, 125 ng) of ^{64}Cu-TETA-octreotide and sacrificed by halothane overdose at time points ranging from 1–48 hr postinjection (n = 4–5 rats/group). The tumor, blood, lung, liver, spleen, kidney, bladder, muscle, heart, bone, bone marrow, adrenals, pancreas, stomach and intestines were removed, weighed and the activity counted on a gamma counter. The bone marrow was collected from the bone by cutting the femur bone in half and removing the bone marrow. Counts were compared to standards, and the percent injected dose per organ (%ID/organ) for each tissue and organ was calculated.

Additionally, seven rats were injected with ^{64}Cu-TETA-octreotide and housed in metabolism cages to determine the %ID excreted in urine at 0.5–1, 2, 3, 5.5 and 24.5 hr and in feces at 24.5 hr postinjection. Bladder dose was calculated by assuming that the %ID excreted in the rat urine was retained in the bladder until the assumed voiding times of 2 and 5.5 hr postinjection. Bladder emptying was assumed to be 90% efficient. The activity remaining in the bladder after the 5.5 hr void was presumed to be retained in the bladder without further voiding.

The assumption was made that the rat biodistribution, determined as the %ID/organ at various time points postinjection, is the same as the human biodistribution for the excised organs. The amount of activity that was not found in the excised organs, urine or feces in the rats was 15.9% and was assumed to be evenly distributed throughout the remainder of the body. Physical decay (and no further tissue clearance) was assumed for time points beyond 48 hr. Time-activity curves were generated for 12 organs. Cumulative activity (μCi-hr or KBq-hr) was determined by integrating the area under the time-activity curves using the computer program KaleidaGraph. Dose estimates were then calculated using standard Medical Internal Radiation Doses (MIRD) techniques, and S values for ^{64}Cu were obtained from MIRDOSE3.

The absorbed dose to the CA20948 tumor was determined from biodistribution data using previously described methods (*13*). Tumor dosimetry for rats carrying CA20948 was also independently determined by PET imaging at various times postinjection of a therapeutic dose of ^{64}Cu-TETA-octreotide. By both methods, the activity was assumed to be homogeneously distributed throughout the tumor. In PET imaging studies, a tumor-bearing rat (250 g) was anesthetized and a membrane port catheter (26 gauge; Abbocath-T, Abbott Labs, Chicago, IL) was placed into the tail vein. After positioning the animal in the PET camera, the catheter was flushed with 2 ml saline. Copper-64-TETA-octreotide [10.31 mCi (389 MBq)] was administered, followed by additional saline (2 ml). Imaging was performed using a CTI ECAT 953B (Knoxville, TN) PET scanner calibrated against a Capintec CRC-12 (Ramsey, NJ) dose calibrator. Images of the entire animal were obtained several times during the first 2 hr following injection and then again at 21 and 49 hr. Calibration of image pixel values is achieved using a previously determined factor to convert pixel counts to μCi/ml (including the positron yield of ^{64}Cu, 19%). This factor was additionally verified by comparing the total injected dose (10.3 mCi) with that measured by a large region of interest (ROI) on all slices through the animal. These factors differed slightly (12%), but as a small amount of activity may have remained in the animal's tail (outside the image field of view), the previously determined factor was used. Standard data corrections were applied to the image data (attenuation, scatter, randoms, deadtime) in addition to a partial volume correction for tumor ROI values (*22*). This latter correction was determined by comparing the size of the lesion (measured after excision) with a previously-determined contrast recovery curve for this scanner. Thus, a correction factor of 1.53 was used to correct the measured contrast (lesion-to-background) to the actual contrast. This corrected peak activity was used to estimate the total accumulated activity in the lesion at various times after injection.

RESULTS

Chemistry

In all experiments, the radiochemical purity of ^{64}Cu-TETA-octreotide was > 90% and the radiochemical purity of ^{64}Cu-TETA was > 99%. The specific activity of the ^{64}Cu-TETA-octreotide in the radiotherapy experiments in tumor-bearing rats ranged from 3000–13,000 Ci/mmol (111–481 GBq/mmol). The specific activity of ^{64}Cu-TETA was 316 Ci/mmol (11.7 GBq/mmol).

Receptor Binding Assays

Copper-64-TETA-octreotide bound to somatostatin receptors in CA20948 membranes with high affinity (n = 3 for all values) (617 ± 98 p*M*) (Fig. 1 for a representative graph). The IC_{50} versus unlabeled Cu-TETA-octreotide was determined to be 0.498 ± 0.039 n*M*. The receptor density in CA20948 membranes (B_{max}) was found to be 352 ± 60 fmol/mg protein, which is similar to the previously reported value of 150 ± 39 fmol/mg protein (*23*).

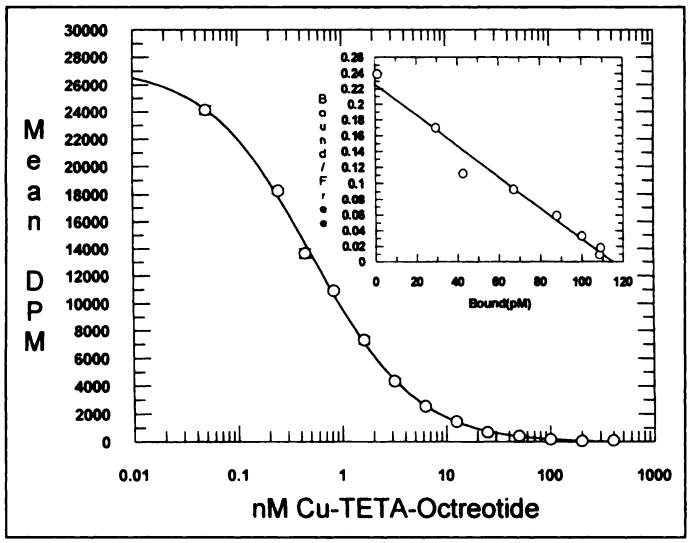

FIGURE 1. Representative displacement graph of ^{64}Cu-TETA-octreotide with unlabeled Cu-TETA-octreotide. The IC_{50} of Cu-TETA-octreotide was 0.498 n*M*. Using LIGAND, scatchard analysis indicated K_d of 507 p*M* [9% coefficient of variance (CV)] and binding capacity (B_{max}) of 384 fmol/mg protein (6% CV).

Single Dose Radiotherapy Experiments

Therapy experiments in tumor-bearing rats were performed initially with single administrations of 5 and 10 mCi of ^{64}Cu-TETA-octreotide. Growth inhibition was not observed at 5 mCi, and only slight growth inhibition was observed at 10 mCi (data not shown). Tumor-bearing rats given one administration of 15 mCi of ^{64}Cu-TETA-octreotide demonstrated significant tumor growth inhibition (Fig. 2) over controls. Figure 2 shows mean tumor growth curves for rats (carrying either smaller or larger tumors) given 15 mCi of ^{64}Cu-TETA-octreotide, as well as rats administered either vehicle, octreotide or 15 mCi of ^{64}Cu-TETA control agents. There was a 4-day delay in the reinitiation of tumor growth (for both larger and smaller tumors) in the treated rats over the controls. The least difference test showed that the tumors from the rats administered the single 15 mCi dose of ^{64}Cu-TETA-octreotide grew significantly slower than the controls ($p < 0.05$), with the average time to reach 3000 mm^3 being 12.3 ± 2.1 days versus 6.6 ± 0.5 days for the controls.

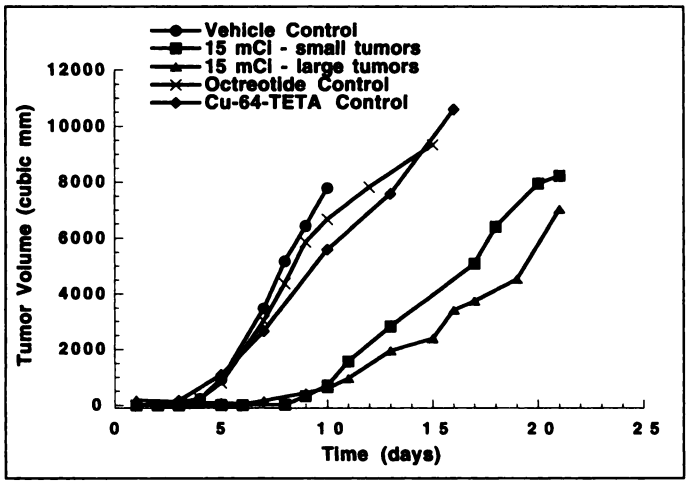

FIGURE 2. Single dose radiotherapy experiment in CA20948 tumor-bearing rats. Rats were administered with either 15 mCi ^{64}Cu-TETA-octreotide (n = 5), vehicle (0.1 *M* NH_4OAc with 0.1% BSA) (n = 5), 1.5 μg octreotide or 15 mCi (555 MBq) ^{64}Cu-TETA (n = 5). In all experiments except one, initial sizes of tumors were small (<20 mg). In one experiment (15 mCi larger tumors) tumor sizes ranged from 103–340 mg (n = 4).

In most of the radiotherapy experiments presented here, rats carrying very small tumors (1–15 mm^3) were administered treatment. In one experiment, tumors were significantly larger (196 mm^3 ± 112 mm^3) on the day of treatment (one dose of 15 mCi). Significant tumor regression occurred in 3 of 4 rats injected with 15 mCi of ^{64}Cu-TETA-octreotide. For example, rat 1 showed a tumor volume decrease from 340 to 85 mm^3, tumor volume in rat 2 dropped from 104 to 1 mm^3 and tumor volume in rat 4 decreased from 230 to 18 mm^3. Rat 3 showed a slight decrease from 110 to 74 mm^3.

Dose Fractionation Radiotherapy Experiments

Four different dose fractionation experiments were performed to determine optimal dose fractionation schedules. Tumor-bearing rats were given a total of 15 mCi divided into either two or three fractions. One experiment was fractionated into three doses on Days 1, 3 and 8, and there was minimal tumor growth inhibition observed over rats treated with control agents (data not shown). This was likely because the tumors had grown significantly after the second dose, and the third dose of 5 mCi was ineffective. An experiment where the dose was fractionated by 12 hr (10 + 5 mCi) showed tumor growth inhibition that was not significantly different than the single 15-mCi dose (data not shown). The most optimal dose fractionation schedules were in two doses, either 1 or 2 days apart. Figure 3 demonstrates the increased growth inhibition attributed to dose fractionation over a single 15 mCi dose. Rats injected with a fractionated dose (either 1 or 2 days apart) showed significantly longer tumor growth inhibition than rats injected with a single 15 mCi dose ($p < 0.05$). The average time for the tumor to reach a volume of 3000 mm^3 was ~18 days for both dose fractionation groups compared with 12.3 days for the single dose group and 6.6 days for the buffer control group.

Toxicity

The treated rats gained weight similarly to the controls throughout the course of the experiment. All blood samples drawn from treated rats had similar hematology and enzyme levels as control rats, except for WBC counts and alkaline phosphatase levels. Figure 4 shows a comparison of WBC counts in rats treated with a single 15 mCi dose of ^{64}Cu-TETA-octreotide, fractionated 15 mCi doses or buffer/BSA (untreated). At 7 days post-treatment, rats treated with one 15 mCi dose of ^{64}Cu-TETA-octreotide showed a lower WBC count than those receiving buffer (4300 ± 880 versus 10,000 ± 2200). By Day 23, the WBC count of one of the four remaining rats had risen to 8000 (the other three rats in that group were killed due to large tumor burden, not toxicity). In a dose fractionation study where 15 mCi was administered in 2 equal doses 1 day apart, there was a less dramatic initial drop at Day 4 in WBCs for the treated versus the control rats (8200 ± 1300 versus 11,400 ± 740), and the WBCs in all rats in the treated group increased to 10,400 ± 1900 by Day 18. Rats treated with one 15 mCi dose of ^{64}Cu-TETA-octreotide showed slightly elevated alkaline phosphatase levels compared to control rats 14 days post-treatment (409 ± 33 U/liter versus 299 ± 53 U/liter); however, there were no significant differences in the other enzyme levels between the two groups.

Normal Organ Dosimetry

Human absorbed doses to normal organs from ^{64}Cu-TETA-octreotide were estimated from biodistribution data in CA20948 tumor-bearing rats. The biodistribution of ^{64}Cu-TETA-octreotide in selected organs at 1, 12 and 48 hr is presented in Table 1. Total activity excreted in the urine by 24.5 hr was 69.2% ± 12.8%, and the total fecal excretion was 7.0% ±

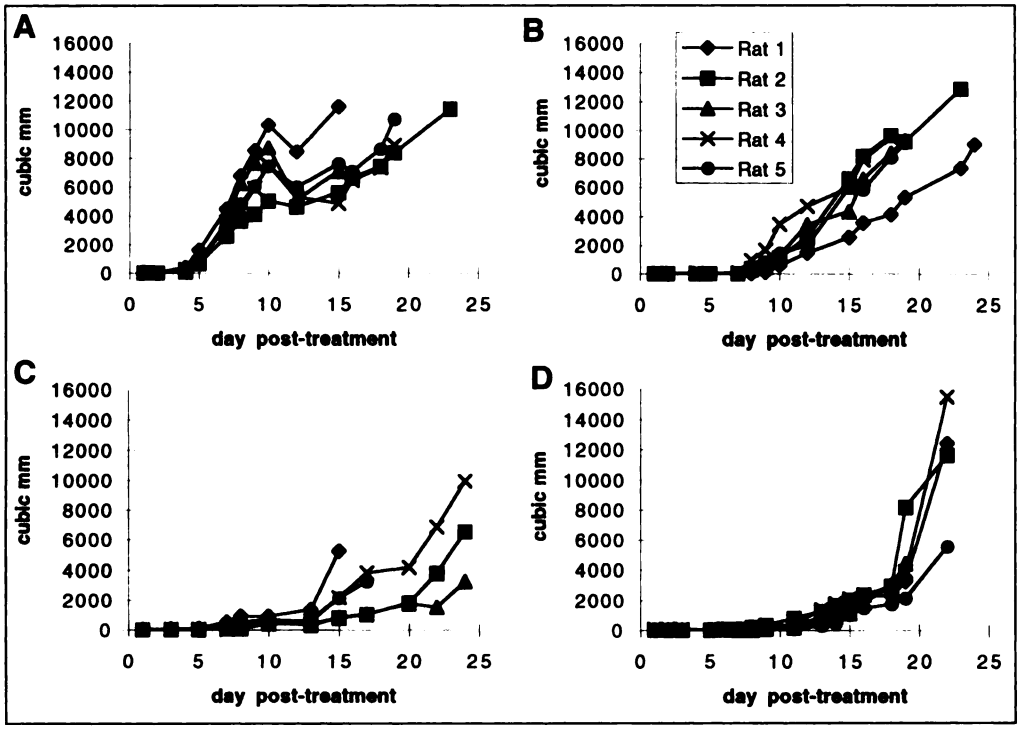

FIGURE 3. Comparison of single dose radiotherapy versus dose fractionation of 15 mCi ^{64}Cu-TETA-octreotide. All radiotherapy experiments were compared to rats injected with vehicle (0.1 *M* NH_4OAc with 0.1% BSA). (A) Vehicle control. (B) Fifteen-millicurie dose (single administration). (C) Dose fractionation (9 + 5 mCi on Days 1 and 3). (D) Dose fractionation (7.5 + 7.5 on Days 1 and 2).

11.8%. Biodistribution data presented in Table 1 shows much higher uptake in the somatostatin-receptor containing tissues (adrenals and pancreas) than what has been previously published (*17*). This is likely due to the different amounts of TETA-octreotide that were administered (125 ng in this study versus 8–20 ng in the previous studies). Breeman et al. (*24*) have shown that the mass of DTPA-octreotide significantly affects the uptake of ^{111}In-DTPA-octreotide in somatostatin-receptor rich tissues in rats, and the trends they observed are consistent with our data.

The estimated human absorbed doses to normal organs are presented in Table 2. The primary and secondary critical organs are the bladder wall (1.12 rad/mCi; 0.30 mGy/MBq) and the lower large intestine (0.86 rad/mCi; 0.23 mGy/MBq). The bladder dose would be highly variable depending on fluid intake and voiding intervals. In humans, this dose can potentially be reduced by either increasing fluid intake or catheterization. The marrow dose is relatively low, 0.070 rad/mCi, compared to the marrow dose for ^{64}Cu-labeled MAb 1A3, which gives a dose of 0.249 rad/mCi (*13*).

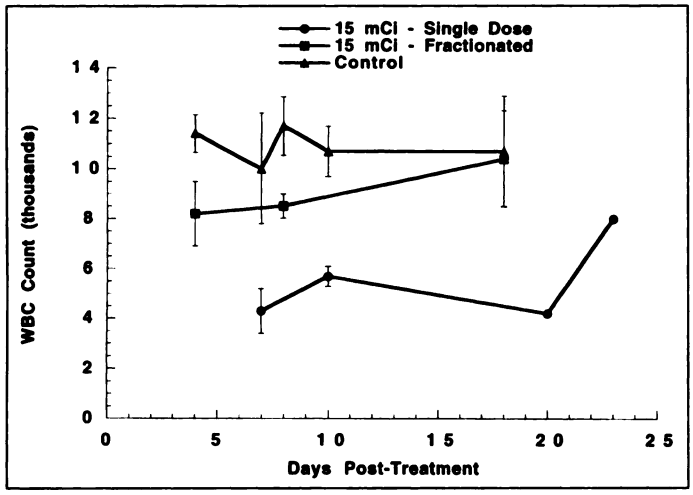

FIGURE 4. White blood cell counts obtained in tumor-bearing rats treated with either a single 15 mCi (555 MBq) dose of ^{64}Cu-TETA-octreotide or two 7.5 mCi doses on Days 1 and 2. These are compared to control rats treated with BSA/buffer. For all groups, n = 4 or 5, except for 15 mCi single dose on Days 20 and 23 where n = 2 and n = 1, respectively.

Tumor Dosimetry

Figure 5 shows the time-activity curve for the CA20948 rat tumor obtained from the PET images. Decay-corrected data were fit with a monoexponential function, then integrated to determine the residence time of the tumor. The S-factor for ^{64}Cu was calculated for the 1.1 g tumor to be 0.266 rad/μCi-hr (0.072 mGy/KBq-hr), assuming a sphere of unit density and complete absorption of nonpenetrating emissions. The absorbed dose to the CA20948 rat tumor calculated from the PET imaging study was 36 rad/mCi (9.7 mGy/MBq) (Note: this is the absorbed dose to a rat tumor and is not an estimated human absorbed dose as reported in Table 2). This compares with a dose of 30.9 rad/mCi (8.4 mGy/MBq) based on conventional dissection measurements and a smaller administered dosage of ^{64}Cu-TETA-octreotide.

TABLE 1
Biodistribution of Copper-64-TETA Octreotide in CA20948 Tumor-Bearing Lewis Rats

Tissue	1 hr %ID/g	12 hr %ID/g	48 hr %ID/g
Blood	0.16 ± 0.058	0.078 ± 0.01	0.11 ± 0.040
Adrenals	6.28 ± 2.09	3.97 ± 0.14	2.33 ± 1.14
Pancreas	2.18 ± 0.80	1.32 ± 0.13	0.52 ± 0.15
ULI	0.21 ± 0.087	0.24 ± 0.022	0.22 ± 0.033
LLI	0.22 ± 0.014	0.69 ± 0.097	0.31 ± 0.076
Kidneys	2.66 ± 0.63	1.66 ± 0.23	0.80 ± 0.11
Liver	0.30 ± 0.089	0.36 ± 0.031	0.37 ± 0.095
Marrow	0.10 ± 0.035	0.23 ± 0.087	0.44 ± 0.28
Spleen	0.16 ± 0.036	0.13 ± 0.038	0.17 ± 0.050
Tumor	1.62 ± 0.40	0.59 ± 0.039	0.38 ± 0.15

TETA = 1,4,8,11-tetraacyclotetradecane-N,N′,N″,N‴-tetraacetic acid; ULI = upper large intestine; LLI = lower large intestine.

The data is presented as %ID/g of tissue ± s.d.; n = 5 for 1- and 12-hr time points; n = 4 for 48 hr group.

TABLE 2
Estimated Human Absorbed Doses to Normal Organs Based on Biodistribution in Tumor-Bearing Lewis Rats

Tissue	Absorbed dose rads/mCi (mGy/MBq)
Bladder wall	1.12 (0.30)
LLI wall	0.86 (0.23)
Kidneys	0.54 (0.15)
Adrenals	0.37 (0.10)
ULI wall	0.16 (0.043)
Pancreas	0.12 (0.032)
Liver	0.10 (0.027)
Marrow	0.07 (0.019)
Spleen	0.047 (0.013)
EDE	0.21 (0.057)

ULI = upper large intestine; LLI = lower large intestine; EDE = effective dose equivalent.

DISCUSSION

The objectives accomplished by this study were fourfold: (a) to further evaluate the potential of ^{64}Cu as a radiotherapeutic radionuclide labeled to a small biomolecule with rapid biological clearance; (b) to determine the effectiveness of a ^{64}Cu-labeled tumor-receptor peptide hormone ligand as a radiotherapeutic agent in tumor-bearing rats administered as both a single and fractionated dose; (c) to estimate the human absorbed doses of ^{64}Cu-TETA-octreotide to normal organs and measure the absorbed dose to the rat tumor that resulted in growth inhibition; and (d) to determine if toxicity resulted in the rat model from therapeutic doses of ^{64}Cu-TETA-octreotide. The results of this study indicated that ^{64}Cu-TETA-octreotide is a potential new radiotherapeutic agent for somatostatin-receptor-positive tumors.

Copper-64 has proven to be a very versatile isotope (*14*) with regard to its applications in both PET imaging (*18,25,26*) and radiotherapy (*13*). Apelgot et al. (*27*) first reported the potential of ^{64}Cu for therapy, and they demonstrated that ^{64}Cu had similar lethality to ^{67}Cu in cell culture despite their different decay schemes. Our group has since reproduced their results, both in vitro (*11,12*) and in vivo (*13*). The on-demand availability of ^{64}Cu from a medical cyclotron (*16*) also makes ^{64}Cu a very attractive isotope for targeted radiotherapy since the technology for producing ^{64}Cu is available to the more than 100 PET centers throughout the world.

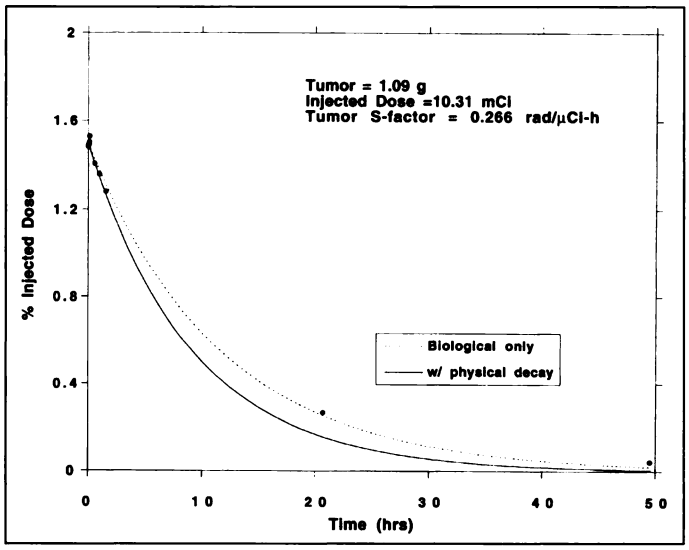

FIGURE 5. Time-activity curve of ^{64}Cu-TETA-octreotide in the CA20948 tumor from PET images of tumor-bearing rat. Rat was injected with 10.3 mCi (389 MBq) and PET images were obtained 6 times between 0–2 hr, and at 21 and 48 hr postinjection. Tumor S-factor was determined to be 0.266 rad/μCi-hr (0.072 mGy/KBq-hr).

Copper-64-TETA-octreotide compares favorably with other radiolabeled somatostatin analogs for radiotherapy that have been published thus far. Distinct tumor growth inhibition was observed in both single doses and fractionated doses. In the therapy experiment with larger tumors, an average of 77% regression of the tumor occurred. Stolz et al. (*8*) evaluated a single injection of 500 μCi ^{90}Y-DTPA-Tyr^3-octreotide in nude mice bearing bearing AR42J rat pancreatic tumors and observed 25% tumor regression, but no permanent tumor growth inhibition. In a more recent abstract, Stolz et al. (*9*) reported complete regression of CA20948 tumors in Lewis rats injected with 2–2.5 mCi (75–100 MBq) ^{90}Y-DOTA-Tyr^3-octreotide. No toxicity or dosimetry was reported in either study. The findings by Stolz et al. (*9*) are especially impressive, since the CA20948 tumor is very aggressive, with a doubling time of 12–24 hr. The CA20948 tumor sizes in the latter study were significantly larger than in the study reported here. The fact that the β^- particle of ^{90}Y has a much longer pathlength than the β^- or the β^+ of ^{64}Cu (8–10 mm versus 2–3 mm, respectively) suggest that ^{90}Y may be better suited to larger tumor burdens, whereas ^{64}Cu appears to be effective with smaller tumors. Further comparisons of these two radionuclides are warranted.

Zamora et al. (*3*) assessed ^{188}Re ($T_{1/2}$ = 17 hr) directly labeled to the somatostatin analog RC-160 as a radiotherapeutic agent for intratumoral injection in nude mice carrying human prostate carcinoma. This study is less comparable to the data presented here since the radiopharmaceutical was administered intratumorally as seven injections of 7.4 MBq (200 μCi) over a 14-day period with a dose to the tumor of 5300 rads per injected dose. This treatment demonstrated complete tumor growth inhibition in 6 of 10 mice, although no hematologic toxicity was reported, nor were any estimated human absorbed doses to normal organs. The practicality of clinically treating somatostatin receptor positive lesions by intratumoral injections remains in question.

The advantages of dose fractionation for RIT have been well documented and evaluated in several RIT studies (*28–32*). In the study presented here with ^{64}Cu-TETA-octreotide, our data confirmed the advantages of dose fractionation, both in improved tumor growth inhibition and reduced toxicity. Fractionation of 15 mCi ^{64}Cu-TETA-octreotide into two doses, either 1 or 2 days apart gave significantly enhanced tumor growth inhibition over administration of a single dose. This could be due to a higher overall uptake of ^{64}Cu-TETA-octreotide in the tumor due in part to the administration of less mass for each fractional dose. Breeman et al. have shown a bell-shaped function of the uptake of ^{111}In-DTPA-octreotide in somatostatin-receptor positive tissues with respect to mass (*24*). Dose fractionation also appeared to change the growth pattern of the tumors. Rats treated with a single dose exhibited a longer period of either tumor regression or nongrowth followed by exponential growth. In rats treated with fractionated doses, the tumor went through a regression or nongrowth period, followed by a period of slower growth and then exponential growth. These differences seemed to be somewhat consistent and were independent of initial tumor size. The decreased toxicity was likely due to the decreased bone marrow suppression, which may be a consequence of distributing the radiation dose over a longer period of time.

Indium-111-DTPA-octreotide, which is approved for human use for gamma scintigraphy of neuroendocrine cancer, is

currently being evaluated as a radiotherapeutic agent in humans. Thus far, two case reports have been published and one brief report on six patients treated with ^{111}In-DTPA-octreotide (*6,7,33*). In both case reports, treatment with ^{111}In-DTPA-octreotide appeared to slow tumor growth, but did not dramatically decrease the size of the tumors, whereas the study by Krenning et al. (*33*) showed three of six patients demonstrated decreased tumor-size post-treatment. The rationale behind ^{111}In as a radiotherapeutic nuclide involves the emission of Auger electrons after radioactive decay by internal conversion. The Auger elections must be emitted in the vicinity of the cell nucleus for lethal DNA damage to occur. There is currently only limited evidence that even a small percentage of ^{111}In-DTPA-octreotide localizes in the cell nucleus (*34*), and results from studies at Washington University show that the majority of ^{111}In activity from ^{111}In-DTPA-octreotide in tumor cells in vivo is associated with lysosomes (*35*).

Preliminary toxicity data were obtained from the treated tumor-bearing rats to assess if the administered dose of 15 mCi, either in a single dose or a fractionated dose, rendered blood, liver or kidney toxicity. With 15 mCi doses of ^{64}Cu-TETA-octreotide, either single or fractionated, there was minimal toxicity observed. The rats gained weight throughout the course of the experiments, and there were no observable signs of toxicity (i.e., >10% weight loss, scruffiness of coat, diarrhea, lethargy). The only toxicity was a significant drop in the WBC counts in the rats treated with a single 15 mCi dose; the WBC count remained low in all but one of the rats throughout the survival period (20 days). The rats treated with two 7.5 mCi doses 24 hr apart experienced only a slight drop in the WBC count, which rebounded after 10 days. Based on these preliminary toxicity results, we anticipate the maximum tolerated dose (MTD) in Lewis rats to be significantly higher than 15 mCi. Due to the large quantities of ^{64}Cu-TETA-octreotide required to determine the MTD in rats, we plan to determine the MTD in mice, followed by future radiotherapy experiments in a tumor-bearing mouse model.

Estimated absorbed doses to the tumor after administration of ^{64}Cu-TETA-octreotide were determined in the same tumor-bearing animal model by both rat biodistribution analysis and PET imaging. The estimated absorbed doses to the tumor in the rat therapy studies were between 465–540 rad. This dose gave significant tumor growth inhibition and, in the case of the larger tumors, caused tumor regression; however, all tumors eventually regrew. Arguments against determining absorbed doses by biodistribution include the administration of nontherapeutic doses (where much smaller amounts of receptor ligand, in this case TETA-octreotide, are used), the requirement of 36 rats to determine the dosimetry and the inability to account for a tumor of dynamic size during the course of the study. We determined an absorbed dose of 36 rad/mCi to the CA20948 tumor in one rat injected with 10.3 mCi of ^{64}Cu-TETA-octreotide from PET imaging data. This calculated absorbed dose to the tumor was very similar to that obtained from biodistribution data (31 rad/mCi). We are verifying this technique with ongoing PET experiments in this animal model. We will also determine the differences in absorbed doses to both normal organs and tumors when nontherapeutic amounts are injected, since the amount of mass of receptor ligand injected is known to significantly affect biodistribution (*24*).

The tumor dose from ^{64}Cu-TETA-octreotide is considerably less (in some cases more than a factor of 10) than what has been reported for radiolabeled MAbs in RIT studies (*36,37*) and is also less than 10 times the tumor dose reported for ^{188}Re-labeled RC-160 (*3*). However, the tumor doses determined in this study are comparable to the absorbed doses obtained for ^{64}Cu-labeled MAb 1A3 in a colorectal tumor-bearing hamster model, where Connett et al. (*13*) calculated 586 rads to the tumor. It is difficult to make direct comparisons between two different tumor-bearing animal models, but our results are encouraging since significant tumor growth inhibition occurred with a tumor dose comparable to other ^{64}Cu-labeled agents. Also, in two separate studies, our data has consistently demonstrated that the tumor doses from ^{64}Cu-radiopharmaceuticals for tumor growth inhibition or regression are significantly lower than what is required for other radiolabeled agents. Direct comparisons of ^{64}Cu-labeled radiopharmaceuticals with corresponding ^{131}I- and ^{90}Y-labeled agents are under way to verify this observation.

CONCLUSION

This study demonstrates the potential of ^{64}Cu-TETA-octreotide as an agent for radiotherapy. The effectiveness of ^{64}Cu-TETA-octreotide in a tumor-bearing animal model, along with minimal observed toxicity and reasonable human absorbed doses to normal organs, is encouraging enough to consider future clinical investigations.

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REFERENCES

1. Wiseman GA, Kvols LK. Therapy of neuroendocrine tumors with radiolabeled MIBG and somatostatin analogs. *Semin Nucl Med* 1995;25:272–278.
2. Smith-Jones PM, Stolz B, Albert R, et al. Synthesis, radiolabelling, and evaluation of DTPA/octreotide conjugates for radiotherapy. *J Lab Compd Radiopharm* 1995;37: 499–501.
3. Zamora PO, Guhlke S, Bender H, et al. Experimental radiotherapy of receptor-positive human prostate adenocarcinoma with ^{188}Re-RC-160, a directly-radiolabeled somatostatin analogue. *Int J Cancer* 1996;65:214–220.
4. Strickland DK, Vaidyanathan G, Friedman HS, Zalutsky MR. Meta[^{131}I]iodobenzylguanidine uptake and meta[^{211}At]astatobenzylguanidine treatment in human medulloblastoma cell lines. *J Neuro-Oncol* 1995;25:9–17.
5. Kwekkeboom DJ, Kho GS, Lamberts SWJ, Reubi J-C, Laissue JA, Krenning EP. The value of octreotide scintigraphy in patients with lung cancer. *Eur J Nucl Med* 1994;21:1106–1113.
6. Krenning EP, Kooij PPM, Bakker WH, et al. Radiotherapy with a radiolabeled somatostatin analogue, ^{111}In-DTPA-Phe^{1}-octreotide: a case history. *Ann N Y Acad Sci* 1994;733:496–506.
7. Fjälling M, Andersson P, Forsell-Aronsson E, et al. Systemic radionuclide therapy using indium-111-DTPA-D-Phe^{1}-octreotide in midgut carcinoid syndrome. *J Nucl Med* 1996;37:1519–1521.
8. Stolz B, Smith-Jones P, Albert R, et al. Somatostatin analogues for somatostatin-receptor-mediated radiotherapy of cancer. *Digestion* 1996;57(suppl 1):17–21.
9. Stolz B, Smith-Jones P, Weckbecker G, et al. Radiotherapy with yttrium-90-labeled DOTA-Tyr^{3}-octreotide in tumor bearing rodents [Abstract]. *J Nucl Med* 1997;38:18P.
10. de Jong M, Breeman WAP, Bernard BF, et al. Evaluation in vitro and in rats of ^{161}Tb-DTPA-octreotide, a somatostatin analogue with potential for intraoperative scanning and radiotherapy. *Eur J Nucl Med* 1995;22:608–616.
11. Connett JM, Anderson CJ, Baumann ML, Welch MJ. Copper-67 and ^{64}Cu labeled monoclonal antibody (MAb) 1A3 as potential agents for radioimmunotherapy [Abstract]. *J Nucl Med* 1993;34:216P.
12. Anderson CJ, Connett JM, Baumann ML, et al. Comparison of ^{67}Cu and ^{64}Cu as potential radionuclides for radiotherapy [Abstract]. *J Nucl Med* 1993;34:134P.
13. Connett JM, Anderson CJ, Guo LW, et al. Radioimmunotherapy with a copper-64-labeled monoclonal antibody: a comparision with ^{67}Cu. *Proc Nat Acad Sci USA* 1996;93:6814–6818.
14. Blower PJ, Lewis JS, Zweit J. Copper radionuclides and radiopharmaceuticals in nuclear medicine. *Nucl Med Biol* 1996;23:957–980.
15. Zinn KR, Chaudhuri TR, Cheng TP, Morris JS, Meyer WA. Production of no-carrier-added ^{64}Cu from zinc metal irradiated under boron shielding. *Cancer* 1994;73:774–778.
16. McCarthy DW, Shefer RE, Klinkowstein RE, et al. The efficient production of high specific activity ^{64}Cu using a biomedical cyclotron. *Nucl Med Biol* 1997;24:35–43.
17. Anderson CJ, Pajeau TS, Edwards WB, Sherman ELC, Rogers BE, Welch MJ. In vitro and in vivo evaluation of copper-64-labeled octreotide conjugates. *J Nucl Med* 1995;36:2315–2325.

18. Dehdashti F, Anderson CJ, Trask DD, et al. Initial results with PET imaging using copper-64-labeled TETA-octreotide in patients with carcinoid tumor [Abstract]. *J Nucl Med* 1997;38:103P.
19. Longnecker DS, Lilja HS, French J, Kuhlmann E, Noll W. Transplantation of azaserine-induced carcinomas of pancreas in rats. *Cancer Lett* 1979;7:197–202.
20. Raynor K, Reisine T. Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. *J Pharm Exp Ther* 1989;251:510–517.
21. Munson PJ, Rodbard D. LIGAND: a versatile computerized approach for characterization of ligand binding systems. *Anal Biochem* 1980;107:220–239.
22. Cutler PD, Schwarz SW, Anderson CJ, et al. Dosimetry of copper-64-labeled monoclonal antibody 1A3 as determined by PET imaging of the torso. *J Nucl Med* 1995;36:2363–2371.
23. Taylor JE, Theveniau MA, Bashirzadeh R, Reisine T, Eden PA. Detection of somatostatin receptor subtype 2 (SSTR2) in established tumors and tumor cell lines: evidence for SSTR2 heterogeneity. *Peptides* 1994;15:1229–1236.
24. Breeman WAP, Kwekkeboom, DJ, Kooij PPM, et al. Effect of dose and specific activity on tissue distribution of indium-111-pentetreotide in rats. *J Nucl Med* 1995;36:623–627.
25. Philpott GW, Schwarz SW, Anderson CJ, et al. RadioimmunoPET: detection of colorectal carcinoma with positron-emitting copper-64-labeled monoclonal antibody. *J Nucl Med* 1995;36:1818–1824.
26. Philpott GW, Dehdashti F, Schwarz SW, et al. RadioimmunoPET (MAb-PET) with copper-64-labeled monoclonal antibody (MAb 1A3) fragments [F(ab')2] in patients with colorectal cancers [Abstract]. *J Nucl Med* 1995;36:9P.
27. Apelgot S, Coppey J, Gaudemer A, et al. Similar lethal effect in mammalian cells for two radioisotopes of copper with different decay schemes, copper-64 and copper-67. *Int J Radiat Biol* 1989;55:365–384.
28. Buchegger F, Pfister C, Fournier K, et al. Ablation of human colon carcinoma in nude mice by ^{131}I-labeled monoclonal anti-carcinoembryonic antigen antibody $F(ab')_2$ fragments. *J Clin Invest* 1989;83:1449–1456.
29. Kwa HB, Verhoeven AHM, Storm J, Vanzandwijk N, Mooi WJ, Hilkens J. Radioimmunotherapy of small cell lung cancer xenografts using iodine-131-labelled anti-Ncam monoclonal antibody 123c3. *Cancer Immunol Immunother* 1995;41:169–174.
30. Buchegger F, Rojas A, Delaloye AB, et al. Combined radioimmunotherapy and radiotherapy of human colon carcinoma grafted in nude mice. *Cancer Res* 1995;55: 83–89.
31. Meredith RF, Khazaeli MB, Liu T, et al. Dose fractionation of radiolabeled antibodies in patients with metastatic colon cancer. *J Nucl Med* 1992;33:1648–1653.
32. DeNardo GL, DeNardo SJ, O'Grady LF, Levy NB, Adams GP, Mills SL. Fractionated radioimmunotherapy of B-Cell malignancies with ^{131}I-Lym-1. *Cancer Res (suppl)* 1990;50:1014s–1016s.
33. Krenning EP, Kooij PPM, Pauwels S, et al. Somatostatin receptors: scintigraphy and radionuclide therapy. *Digestion* 1996;57(suppl 1):57–61.
34. Andersson P, Forssell-Aronsson E, Johanson V, et al. Internalization of indium-111 into human neuroendocrine tumor cells after incubation with indium-111-DTPA-D-Phe^1-octreotide. *J Nucl Med* 1996;37:2002–2006.
35. Duncan JR, Stephenson MT, Wu HP, Anderson CJ. Indium-111-diethylenetriaminepentaacetic acid-octreotide is delivered in vivo to pancreatic, tumor cell, and hepatocyte lysosomes. *Cancer Res* 1997;57:659–671.
36. Sharkey RM, Pykett MJ, Siegel JA, Alger EA, Primus FJ, Goldenberg DM. radioimmunotherapy of the GW39 human colonic tumor xenograft with I-131-labeled murine monoclonal antibody to carcinoembryonic antigen. *Cancer Res* 1987;47:5672–5677.
37. Blumenthal RD, Sharkey RM, Kashi R, Goldenberg DM. Comparison of therapeutic efficacy and host toxicity of two different ^{131}I-labelled antibodies and their fragments in the GW-39 colonic cancer xenograft model. *Int J Cancer* 1989;44:292–300.

Evaluation of the Detectability of Breast Cancer Lesions Using a Modified Anthropomorphic Phantom

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During the development and characterization of imaging technology or new imaging protocols, it is usually instructive to perform phantom experiments. Often, very simplified forms of the realistic patient anatomy are used that may be acceptable under certain conditions; however, the implications for patient studies can be misleading. This is particularly true in breast and axillary node imaging. The complexities presented by the anatomy, variable object scatter, attenuation and inhomogeneous distribution of activity in this upper thoracic region provide a significant challenge to the imaging task. **Methods:** A tissue-equivalent anthropomorphic phantom of the thorax (Radiology Support Devices, Inc., Long Beach, CA) containing fillable cavities and organs was modified for the studies. The phantom was filled with realistic levels of FDG activity and scanned on a Siemens ECAT HR+ whole-body PET scanner. Breast attachments containing 2.0- and 2.55-cc lesions with lesion-to-background ratios of 5:1 and 7:1, respectively, were imaged. Scatter and attenuation effects were analyzed with various experimental setups. A lymph node experiment and a multibed position whole-phantom scan also were performed to illustrate the extent to which the phantom represents the human thorax. **Results:** Regions of interest were drawn on the lesions as well as the background breast tissue in all studies. It was found that the signal-to-noise ratio decreased 65% when a more realistic phantom (lesions plus breasts plus thorax, all containing activity) was used, as compared to a simple phantom (lesions plus breasts containing activity; no thorax), due to the effects of increased scatter and attenuation. A 23% decrease in the contrast also was seen from the scan of the more realistic phantom due to surrounding activity from nearby organs such as the heart, as well as an increase in the volume of attenuating media. **Conclusion:** This new phantom allows us to more realistically model the conditions for breast and lymph node imaging, leading to preclinical testing that will produce results that better approximate those that will be found in vivo. The phantom will be a valuable tool in comparing different imaging technologies, data collection strategies and image reconstruction algorithms for applications in breast cancer using PET, SPECT or scintimammography systems.

Key Words: phantom; fluorodeoxyglucose PET; breast cancer; breast; lymph nodes

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It was predicted that 184,300 new invasive cases of breast cancer among women in the U.S. would be diagnosed in 1996 (*1*). Breast cancer is the second leading cause of cancer death in women. Currently, mammography and physical breast examination are the two most effective techniques available for screening potential breast cancer patients.

To improve the quality of care and management of breast cancer patients, in conjunction with screening mammography and other established techniques, there has been a large impetus toward the development of new techniques, altered paradigms and dedicated imaging systems for breast cancer. Scintimammography, lymphoscintigraphy and positron emission mammography, as well as other dedicated imaging techniques and

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