

Radiotherapy and Dosimetry of ^{64}Cu -TETA-Tyr³-Octreotate in a Somatostatin Receptor-positive, Tumor-bearing Rat Model¹

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ABSTRACT

^{64}Cu [$T_{1/2} = 12.8$ h; $\beta^+ = 0.655$ MeV (19%); $\beta^- = 0.573$ MeV (40%)] has shown promise as a radioisotope for targeted radiotherapy. It has been demonstrated previously that the somatostatin analogue ^{64}Cu -TETA-octreotide (^{64}Cu -TETA-OC, where TETA is 1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid) significantly inhibited the growth of somatostatin receptor-positive CA20948 rat pancreatic tumors in Lewis rats (C. J. Anderson *et al.*, *J. Nucl. Med.*, 39: 1944–1951, 1998). In this study, we evaluated the radiotherapeutic efficacy of a new ^{64}Cu -labeled somatostatin analogue, ^{64}Cu -TETA-Tyr³-octreotate (^{64}Cu -TETA-Y3-TATE), in CA20948 tumor-bearing rats. A single dose of 15 mCi (555 MBq) of ^{64}Cu -TETA-Y3-TATE was shown to be more effective in reducing tumor burden than the same dose of ^{64}Cu -TETA-OC. In multiple dose experiments, tumor-bearing rats were administered three doses of either 10 or 20 mCi (370 or 740 MBq) of ^{64}Cu -TETA-Y3-TATE at 48-h intervals. Rats given 3×10 mCi (3×370 MBq) showed extended mean survival times compared with rats given a single dose; however, no complete regressions occurred. Complete regression of tumors was observed for all rats treated with 3×20 mCi (3×740 MBq), with no palpable tumors for ~ 10 days; moreover, the mean survival time of these rats was nearly twice that of controls. Toxicity was determined by physical appearance and hematological and enzyme analysis, which revealed no overt toxicity and

only transient changes in blood and liver chemistry. Absorbed dose estimates showed the dose-limiting organ to be the kidneys. The radiotherapy results, along with absorbed dose estimates to target and clearance organs, confirm that ^{64}Cu -labeled somatostatin analogues warrant continued consideration as agents for targeted radiotherapy.

INTRODUCTION

Somatostatin analogues have been investigated for utility in scintigraphic and PET³ imaging of cancer in humans. For example, ^{111}In -pentetreotide (^{111}In -DTPA-octreotide; Refs. 1 and 2) has been approved for routine clinical use in the diagnosis of neuroendocrine cancer in the United States and Europe. Somatostatin analogues have also been labeled with other radionuclides and evaluated as possible radiotherapeutic agents. Targeted radiotherapy studies have been performed in animal models with somatostatin analogues labeled with ^{90}Y (3–5), ^{111}In (6), and ^{64}Cu (7) with varying degrees of success, and of these agents, ^{111}In -DTPA-octreotide (8–10) and ^{90}Y -DOTA-Tyr³-octreotide (^{90}Y -SMT 487, ^{90}Y -DOTATOC, or ^{90}Y -DOTA-Y3-OC; Ref. 11) are being investigated in ongoing clinical radiotherapy trials.

Improvement in target tissue uptake of radiolabeled somatostatin analogues has been the focus of a number of studies. It has been shown that substitution of a tyrosine (Y) for phenylalanine (F) in the 3-position and changing the C-terminus from an alcohol to a carboxylic acid increases uptake of the peptide in receptor-rich tissues (12–14). This has been confirmed by our own studies, where ^{64}Cu -TETA-Tyr³-octreotate (^{64}Cu -TETA-Y3-TATE; Fig. 1) demonstrated significantly greater uptake in somatostatin-rich tissues in two tumor-bearing animal models (Lewis rats bearing CA20948 tumors and severe combined immunodeficient mice bearing AR42J tumors) compared with ^{64}Cu -TETA-octreotide (^{64}Cu -TETA-OC; Fig. 1; Refs. 15 and 16).

^{64}Cu [$T_{1/2} = 12.8$ h; $\beta^+ = 0.655$ MeV (19%); $\beta^- = 0.573$ MeV (40%)] has diverse applications in radiopharmaceutical chemistry for PET imaging (17) as well as therapy (7, 18). Moreover, ^{64}Cu can be produced on demand in high yield and in high specific activity on a small biomedical cyclotron (19), making it a radionuclide available to many medical institutions.

We report herein an investigation into the radiotherapeutic potential of ^{64}Cu -TETA-Y3-TATE in CA20948 tumor-bearing

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³ The abbreviations used are: PET, positron emission tomography; Y3, tyrosine-3; TETA, 1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid; OC, octreotide; TATE, octreotate; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ID/g, injected dose per gram; MIRD, medical internal radiation dose; ROI, region of interest.

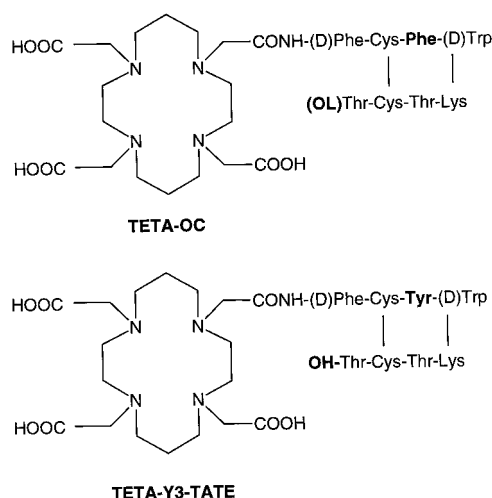


Fig. 1 Structures of TETA-OC (top) and TETA-Y3-TATE (bottom).

rats, a model of somatostatin receptor-positive pancreatic cancer. The therapeutic efficacy of ^{64}Cu -TETA-Y3-TATE was compared with ^{64}Cu -TETA-OC and control agents. In addition, hematological, liver, and kidney assays were performed to evaluate the potential toxicity of the ^{64}Cu -TETA-Y3-TATE. Human absorbed dose estimates for ^{64}Cu -TETA-Y3-TATE were calculated from both rat biodistribution data and PET imaging of a baboon.

MATERIALS AND METHODS

Synthesis of ^{64}Cu -TETA-OC and ^{64}Cu -TETA-Y3-TATE. ^{64}Cu was produced on a biomedical cyclotron at the Washington University School of Medicine by methods reported previously (19). ^{64}Cu -TETA-OC and ^{64}Cu -TETA-Y3-TATE were prepared according to literature methods (15, 16, 20), in specific activities ranging from 1.25 to 2.5 mCi/ μg (46–93 MBq/ μg).

Animal Models. All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee. The rat pancreatic tumor CA20948 (21) was obtained from the Tumor Bank at Biomeasure, Inc. (Hopkinton, MA). Adult male Lewis rats (230–290 g) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The CA20948 cell line was maintained by serial passage in animals. In rat experiments, male Lewis rats were injected with a 1-mm³ tumor section of CA20948 tumor 10 days prior to treatment as described previously (7).

Radiotherapy Experiments (Single Dose). Tumor-bearing rats (tumor volume, 0.3–1.0 cm³) were injected with one dose of 200 μl of 0.1 M NH₄OAc (buffer), 15 μg of TETA-Y3-TATE, 15.5 mCi (574 MBq) of ^{64}Cu -TETA-OC, or 15.5 mCi (574 MBq) of ^{64}Cu -TETA-Y3-TATE. The tumor volume was measured every 1–3 days (using calipers), and rats were sacrificed by administration of Metofane, followed by cervical dislocation, when the tumors reached a volume of ~ 10 cm³ or became ulcerated.

Radiotherapy Experiments (Multiple Dose). Two different multiple dose protocol experiments were carried out. In the first experiment, one group of tumor-bearing rats (tumor volume, 0.3–1.5 cm³) received three 10-mCi (370 MBq) doses of ^{64}Cu -TETA-Y3-TATE, with a control group receiving equivalent masses of unlabeled TETA-Y3-TATE. Rats were injected 10, 12, and 14 days after implantation of tumor cells. The second experiment was identical, except that the treated group of rats received three 20-mCi (740 MBq) doses of ^{64}Cu -TETA-Y3-TATE 10, 12, and 14 days after implantation, and the control group received equivalent masses of unlabeled TETA-Y3-TATE on those days. Tumor volumes were measured, and animals were sacrificed in a manner identical to that described for the single-dose radiotherapy experiments.

Blood Chemistry. Hematological, liver, and kidney chemistries were studied for the group of tumor-bearing rats that received 3×20 mCi. These results were compared with those obtained from rats treated with control agents. In each group, anesthetized rats were weighed, and blood was removed by cardiac puncture during the posttherapy survival period. Toxicity analysis was performed by the Diagnostic Services Laboratory in the Department of Comparative Medicine at Washington University School of Medicine. The hematology analysis included WBC counts, RBC counts, platelet counts, as well as measurement of hemoglobin, hematocrit, and differential WBCs. Liver and kidney analysis included blood urea nitrogen, creatinine, ALP, ALT, and AST.

Effect of Specific Activity on Biodistribution of Radio-labeled Peptide. To determine the effect of peptide mass on the biodistribution of ^{64}Cu -TETA-Y3-TATE, groups of CA20948 tumor-bearing rats ($n = 5$) were coinjected with 10 ng of ^{64}Cu -TETA-Y3-TATE (5 μCi , 0.2 MBq) and a known mass of TETA-Y3-TATE to give final injectates of 10, 50, 100, 500, 1000, and 5000 ng. All animals were sacrificed at 1 h, and % ID/g and % ID/organ values of selected tissues and organs were determined.

Rat Dosimetry. The estimated human absorbed doses of ^{64}Cu -TETA-Y3-TATE to normal organs were obtained using biodistribution data in CA20948 tumor-bearing rats, according to methods described previously (7). ^{64}Cu -TETA-Y3-TATE (35 μCi , 1.3 MBq) was injected i.v., and the rats were euthanized by Metofane overdose and cervical dislocation at 1, 3, 6, 12, 24, 36, and 48 h after injection. The rats for the 48-h time point were housed in metabolism cages to determine % ID excreted in urine and feces at 1, 3, 6, 17, 24, 43, and 48 h. Time-activity curves were generated for 12 organs. Cumulative activity ($\mu\text{Ci}\cdot\text{h}$ or kBq $\cdot\text{h}$) was determined by numerically integrating the area under the time-activity curves. Human dose estimates were then calculated using standard MIRD techniques, and S-values (mean absorbed dose per unit cumulative activity) for ^{64}Cu were obtained from the MIRDSE3 program (22). Bone activity was assumed to be distributed equally between the trabecular bone and cortical bone. The absorbed dose to the CA20948 rat tumor was determined from biodistribution data using methods described previously (18). Briefly, time-activity data were determined by combining the tumor data from different time points after injection. Each tumor was excised and weighed, and an S-value was calculated for the average tumor size for each time point, assuming a spherical tumor of unit density. Nonpenetrat-

ing emissions of ^{64}Cu were assumed to be completely absorbed in the tumor. For penetrating emissions, appropriate specific absorbed fractions for the 511 and 1340 keV photons of ^{64}Cu were used. This approach assumes that tumors of similar size in different animals demonstrate similar uptake characteristics, and the resulting absorbed dose is an average of that absorbed by each tumor.

Baboon Dosimetry. The biodistribution of ^{64}Cu -TETA-Y3-TATE was also determined in a 25-kg male baboon by PET imaging. PET imaging was performed using a Siemens/CTI ECAT EXACT PET system (CTI PET Systems, Knoxville, TN) to determine the biodistribution of 4.6 mCi (170.2 MBq) of ^{64}Cu -TETA-Y3-TATE over the first 18 h after injection. Images of the animal's torso were acquired at 30-min intervals from 0 to 3 h and then again at 18 h after injection. The baboon was anesthetized for the first 3 h and then repositioned to approximately the same position the following day.

Activity concentration values were derived from the PET images, which were calibrated previously against the same dose calibrator (Capintec, Ramsey, NJ) used to assay the injected dose. Corrections for photon attenuation, random coincidences, deadtime, and scatter were applied. Images were reconstructed with filtered backprojection and modest smoothing (Hann, cut-off 0.3 pixels⁻¹), so that most organs could be clearly identified. ROIs were drawn over liver, spleen, kidneys, bladder, blood pool, red marrow, and muscular soft tissue and were used to estimate total organ accumulations of the compound. Blood activity was taken to be the average of the maximum pixels in five to six adjacent slices through the left ventricle, which was necessary to avoid partial volume effects in the moving heart. Liver activity was taken as the average value in a large ROI centered in the liver and averaged > five to six slices. Kidney activity, although trapped primarily in the renal cortex, was taken as the average value inside a ROI outlining the entire kidney and was assumed to be uniformly distributed in the organ. ROI values were decay-corrected to the time of injection, extrapolated where necessary by standard human organ and blood volumes, and then normalized to the baboon's weight (23). By comparison with the total injected activity, the % ID to each organ was determined. Bone marrow activity was derived from blood pool activity according to the model of Siegel *et al.* (24) using a partition fraction of 0.3.

Time-activity curves were generated from the PET ROI results for the seven organs. Each was fit with a biexponential function (Fig. 2) and then integrated numerically to determine the residence time of the activity in each organ. The results accounted for 90–99% of the injected activity over the imaging period, with the rest being included as “missing” and assigned to the MIRD category “remainder-of-body.” Thus, the missing fraction was assumed to be distributed uniformly in the body. The “residence time,” or accumulation-time product, can be determined for each relevant organ by integrating the time-activity curve either analytically, from 0 to ∞ , or numerically over a suitably long interval. We used a numerical integration from 0 to 48 h. The absorbed radiation dose to a given organ was calculated as the sum of the products of the residence times and the tabulated S-values for ^{64}Cu in a standard human geometry. Because the bladder residence time depends primarily on the pattern of voiding, we have calculated a conservative estimate

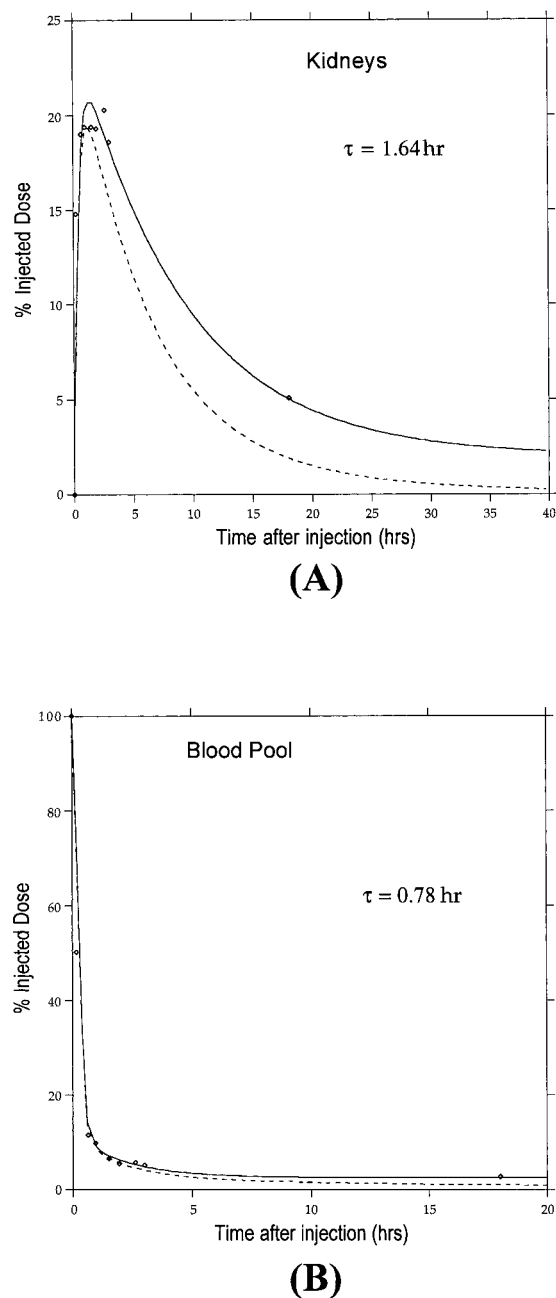


Fig. 2 Time-activity curves for kidneys (A) and blood (B) from PET images of a baboon injected with ^{64}Cu -TETA-Y3-TATE. Data points in the upper curve (solid line) are corrected for physical decay, forming the basis for the biexponential fit. The lower curve is the fitted curve with physical decay included, from which the residence time, τ , is derived by numerical integration.

assuming no excretion, as well as a more realistic estimate using the dynamic bladder model available in the MIRDSE3 software package, with a voiding interval of 4 h.

Statistical Analysis. To determine statistical significance in the biodistribution studies, a Tukey's Studentized Range (HSD) Test was performed with $P < 0.05$ being consid-

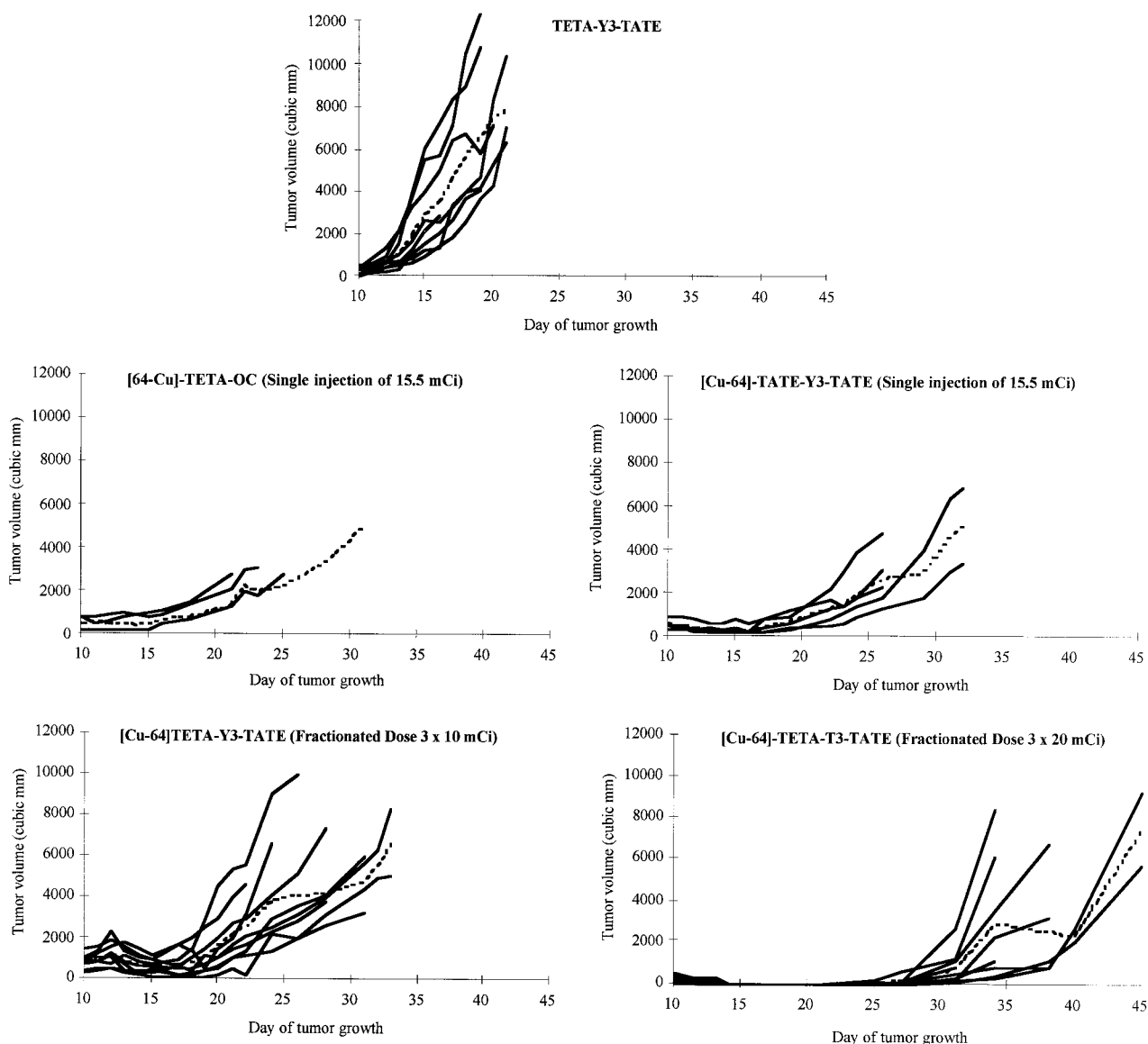


Fig. 3 Radiotherapy experiments in CA20948 tumor-bearing rats. Comparison of tumor growth in control rats and rats treated with ^{64}Cu -TETA-OC and ^{64}Cu -TETA-Y3-TATE. —, individual rats; ---, mean values.

ered significantly different. In the therapy studies, Tukey's Studentized Range (HSD) Test, ANOVA, Scheffe's test, and a Least Difference Test were performed to compare single-dose and multiple-dose protocols and to compare these with data obtained from control rats. Comparison was made on the length of time it took the tumor to reach 10,000 mm^3 in size or the time to ulceration.

RESULTS

Radiotherapy Experiments (Single Dose). The results from all of the radiotherapy studies undertaken in CA20948 tumor-bearing rats are shown in Fig. 3. All rats that received a single 15.5-mCi dose of ^{64}Cu -TETA-Y3-TATE showed significant reduction in tumor volume (29–73%) over the following

7-day period, taking 28.4 ± 3.29 days for the tumor volume to reach $>10,000 \text{ mm}^3$ or to ulcerate after tumor implant. Inhibition of tumor growth and reduction of tumor size after a single dose of ^{64}Cu -TETA-Y3-TATE were more apparent ($P < 0.05$) than that observed in animals receiving an equal dose of ^{64}Cu -TETA-OC (0–35% reduction over 7 days). There was no tumor growth inhibition in the control groups, and the time it took for the tumors to reach $>10,000 \text{ mm}^3$ or to ulcerate (20.71 ± 2.06 days for TETA-Y3-TATE and 19.44 ± 1.67 days for buffer) were significantly lower than the groups that received a single dose of ^{64}Cu -TETA-Y3-TATE ($P < 0.05$). The single 15.5-mCi dose of ^{64}Cu -TETA-Y3-TATE reduced and inhibited the growth of the CA20948 tumor for over 10 days before normal growth resumed. There was no weight loss or appearance of

toxicity with ^{64}Cu -TETA-Y3-TATE at the 15.5-mCi dose. It is important to note that tumors that regrew after remission often ulcerated at an earlier stage than those in controls groups.

Radiotherapy Experiments (Multiple Dose). In both multiple dose experiments, the control groups (10–15 μg of unlabeled TETA-Y3-TATE) showed unrestricted growth of the tumor (mean survival time, 19.25 ± 2.63 days). All rats receiving the 3×10 mCi dose regimen of ^{64}Cu -TETA-Y3-TATE showed tumor growth inhibition and a decrease in tumor volume of 36–81%. The smallest tumor sizes were recorded between days 16 and 18 after implantation (0.07–0.60 cm^3). In the treated rats, it took an average of 28.44 ± 3.91 days for the tumors to reach $>10,000$ mm^3 or to ulcerate after implant. In rats receiving a 3×20 mCi dose regimen, there were no palpable tumors in any of the animals at day 14 after tumor implantation. Complete remission and disappearance of the tumor was observed for 10 days. On day 25 after the implant, tumors began to reappear and continued to grow more slowly than before the therapy, until the animals had to be sacrificed 38.22 ± 4.27 days after tumor implant. By using the Tukey's Studentized Range Test, we found that the time it took the tumors to reach $>10,000$ mm^3 in size or to ulcerate in the rats receiving a 3×20 mCi dose regimen was significantly higher than all other groups examined in all other therapy and control protocols ($P < 0.05$).

Blood Chemistry and Physical Appearance. Toxicity was determined in rats receiving 3×20 mCi of ^{64}Cu -TETA-Y3-TATE by monitoring weight and gross physical appearance, as well as hematological and liver and kidney function. The mean weight of the treated rats increased similarly to that of the control rats, and they maintained a healthy physical appearance (with no sign of scruffy coat or diarrhea) over the experimental period. Blood chemistries were compared with baseline levels obtained from the control rats. The mean WBC count decreased to 25–50% of the control group level at day 12 after the tumor implant ($5,500 \pm 3,630/\text{mm}^3$ versus $13,600 \pm 1,170/\text{mm}^3$). The transient drop in WBCs remained constant until day 15 ($7,500 \pm 1,690/\text{mm}^3$) and then was seen to recover to baseline levels by day 34 ($9,530 \pm 2,290/\text{mm}^3$) after the tumor implant. The differential WBCs showed a transient elevation in segregated neutrophils to a maximum of 407% of controls after 17 days ($61.0 \pm 5.29\%$ versus $15.0 \pm 1.63\%$), with a transient decrease in lymphocytes to a minimum of 45% of controls at day 17 ($37.0 \pm 4.55\%$ versus $82.0 \pm 2.16\%$). Both segregated neutrophils and lymphocyte levels were seen to recover to baseline levels by day 34 ($23.75 \pm 9.03\%$ and $71.8 \pm 8.66\%$, respectively). No significant changes in RBCs or hemoglobin or hematocrit levels were noted. Platelet levels initially elevated to 138% of controls up to day 17 ($11,27.50 \pm 106.59 \times 10^3/\text{mm}^3$ versus $815.25 \pm 87.43 \times 10^3/\text{mm}^3$) and then decreased dramatically to 35% of controls by day 25 ($282.25 \pm 119.69 \times 10^3/\text{mm}^3$). Baseline platelet levels were reached by day 34 ($969.00 \pm 151.39 \times 10^3/\text{mm}^3$). The levels of kidney enzymes, blood urea nitrogen and creatinine, did not change significantly over the observation period. Levels of ALP, ALT, and AST were all seen to decrease (65.1, 51.9, and 49.9% of controls, respectively) by day 17. Both ALP and AST recovered to $>75\%$ of baseline levels by day 20, and ALP, ALT and AST all returned to baseline levels by day 34.

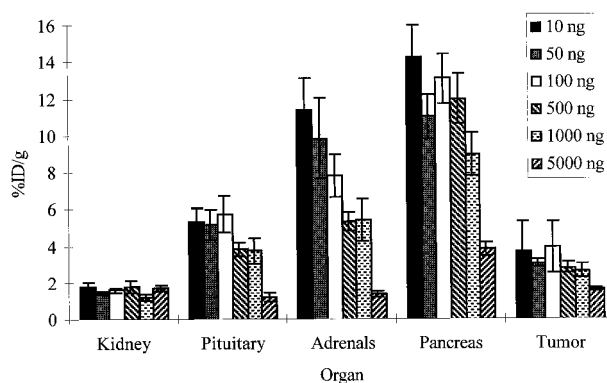


Fig. 4 Biodistribution at 1 h after injection of 5 μCi (0.2 MBq) of ^{64}Cu -TETA-Y3-TATE in CA20948-bearing rats at doses of 10, 50, 100, 500, 1000, and 5000 ng of peptide. Bars, SD.

Effect of Specific Activity on Biodistribution of Radio-labeled Peptide. The uptake of 5 μCi (0.2 MBq) of ^{64}Cu -TETA-Y3-TATE, diluted with different masses of unlabeled TETA-Y3-TATE, in receptor-rich organs and the kidney at 1 h after injection is shown in Fig. 4. With a coinjection of 5000 ng of peptide, uptake decreased significantly in receptor-positive tissues ($P < 0.05$). The uptake in the pituitary showed a 4-fold decrease ($5.40 \pm 0.74\%$ ID/g for 10 ng versus $1.24 \pm 0.24\%$ ID/g for 5000 ng; $P < 0.05$), the adrenals displayed an 8-fold decrease ($11.41 \pm 0.167\%$ ID/g for 10 ng versus $1.40 \pm 0.19\%$ ID/g for 5000 ng; $P < 0.05$), the uptake in the pancreas was nearly 4-fold lower ($14.24 \pm 1.74\%$ ID/g for 10 ng versus $3.84 \pm 0.41\%$ ID/g for 5000 ng; $P < 0.05$), and the tumor uptake was over 2-fold lower ($3.69 \pm 1.68\%$ ID/g for 10 ng versus $1.64 \pm 0.06\%$ ID/g for 5000 ng; $P < 0.05$) at the highest mass injected. Other nontarget organs showed no significant decreases in uptake.

Dosimetry. Human absorbed dose estimates to normal organs, calculated from rat biodistribution data and baboon PET imaging, are shown in Table 1. The absorbed dose to the kidneys for ^{64}Cu -TETA-Y3-TATE, based on rat biodistribution, was 0.445 rad/mCi (0.120 mGy/MBq). By comparison, baboon PET image data showed a peak of 20% ID in the kidneys, with fairly rapid clearance, giving an absorbed dose of 1.25 rad/mCi (0.337 mGy/MBq). The intestinal tract also showed distinctly different uptake results from the rat and baboon experiments. There was virtually no uptake in the bowel of the baboon, and only a small fraction (0.9% ID) cleared via the hepatobiliary system. Table 1 also shows the effect of the dynamic bladder model on the dose to the urinary bladder wall in the PET imaging study. In the CA20948 rat model, the average absorbed dose to the tumor was calculated to be 40.8 rad/mCi (11.0 mGy/MBq) for a single injection of ^{64}Cu -TETA-Y3-TATE.

DISCUSSION

^{64}Cu -TETA-OC is presently being evaluated clinically at Washington University School of Medicine for the detection of neuroendocrine cancer by PET (25) and was investigated for therapeutic potential in a rodent tumor model (7). ^{64}Cu -TETA-Y3-TATE has been shown to have a higher affinity for the

Table 1 Absorbed radiation doses resulting from administration of ^{64}Cu -TETA-Y3-TATE, determined from rat biodistribution and PET imaging of a baboon

Organ	Rat rad/mCi (mGy/MBq)	Baboon rad/mCi (mGy/MBq)
Kidneys	0.445 (0.120)	1.25 (0.337)
Liver	0.108 (0.029)	0.39 (0.107)
Gallbladder		0.49 (0.132)
Red marrow	0.069 (0.018)	0.038 (0.009)
Spleen	0.053 (0.014)	0.35 (0.095)
Pancreas	0.145 (0.039)	0.16 (0.043)
Adrenals	0.616 (0.166)	0.059 (0.016)
Upper large intestine wall	0.244 (0.066)	0.035 (0.010)
Small intestine	0.131 (0.035)	0.044 (0.012)
Lower large intestine wall	1.030 (0.278)	0.059 (0.016)
Urinary bladder	0.785 (0.212)	2.82 (0.763) ^a /0.38 (0.103) ^b
Total body	0.040 (0.011)	0.063 (0.017)

^{a,b} Urinary bladder dose calculated assuming: ^a no excretion; and ^b using the dynamic bladder model of Cloutier *et al.* (37).

somatostatin receptor than ^{64}Cu -TETA-OC both *in vitro* and *in vivo*; *in vitro*, IC_{50} s for the binding of Cu-TETA-Y3-TATE and Cu-TETA-OC to CA20948 pancreatic tumor cell membranes were 0.250 ± 0.05 nM and 0.498 ± 0.039 nM, respectively; *in vivo*, this increased affinity for somatostatin receptors was shown by a 2-fold uptake of ^{64}Cu -TETA-Y3-TATE over ^{64}Cu -TETA-OC into CA20948 tumors over 1 h (15). The aim of the present study was to determine the radiotherapeutic efficacy of the superior analogue, ^{64}Cu -TETA-Y3-TATE, in a tumor-bearing rodent model, to evaluate the toxicity of the agent, and to calculate human absorbed doses from both rodent biodistribution and primate imaging. The results obtained in this investigation strongly suggest that ^{64}Cu -TETA-Y3-TATE may be superior to ^{64}Cu -TETA-OC and has potential for targeted radiotherapy of neuroendocrine cancer in humans.

From the single-dose radiotherapy experiment (1×15.5 mCi), it is evident that ^{64}Cu -TETA-Y3-TATE is at least as effective as ^{64}Cu -TETA-OC in effecting greater tumor regression and may possibly be more effective. In the first multiple dose protocol using ^{64}Cu -TETA-Y3-TATE (3×10 mCi), the tumor burden decreased dramatically with an extended time for the tumor burden to reach $>10,000$ mm³ or ulcerate in the treated animals compared with the control groups. In the second multiple dose regimen (3×20 mCi), there were no palpable tumors in the treated group for an extended period of time (~ 10 days). Moreover, the time for the tumor burden to reach $>10,000$ mm³ or to ulcerate in these rats was nearly twice that of the control groups ($P < 0.05$). All of the statistical analysis performed confirmed that the survival time of the rodents was dependent on the dose administered. It was shown that the 3×20 mCi multiple dose regimen was more effective in tumor regression than single-dose administration and the first multiple dose protocol (3×10 mCi). The advantages of multiple dose protocols over a single dose have precedence in radioimmunotherapy studies (26, 27) and radiotherapy with peptides (7, 28) and include significant reduction of the tumor burden with decreased toxicity. A multiple dose regimen also has the advantage of delivering a consistent amount of tolerable radiation over

an extended period to the tumor, while allowing intermittent recovery of nontarget tissues. The decreased toxicity is often attributable to decreased bone marrow suppression, which is the result of delivery of multiple smaller radiation doses over an extended treatment period.

The CA20948 rat pancreatic tumor is extremely aggressive, with a doubling time of 12–36 h. Stolz *et al.* (4) recently reported complete eradication of CA20948 tumors in 71% of rats treated with 10 mCi/kg (370 MBq/kg) of ^{90}Y -SMT 487 (^{90}Y -DOTA-Y3-OC), with no observable side effects. No absorbed dose measurements to the tumor or normal organs or specific blood chemistry/toxicity data were reported, however. The efficacy of ^{90}Y -SMT 487 has been reported in three patients (11), and this agent is presently in Phase I clinical trials in patients with somatostatin receptor-positive malignancies (3, 29). ^{90}Y has a mean β energy of 0.9 MeV with a maximum energy of 2.27 MeV and a maximum particle range of about 11 mm in tissue, making it an appropriate radionuclide for large tumor burdens. ^{64}Cu emits a 0.58-MeV β^- particle (40%), a 0.66-MeV β^+ particle (19%), and a γ photon of 1.34 MeV (0.5%), yielding a mean range of penetrating radiation of ~ 1.4 mm in tissue; therefore, ^{64}Cu emissions are more suitable for smaller tumor masses. In the ^{90}Y study, the tumor sizes were $12,805 \pm 1140$ mm³ at the time of injection (4). In our studies, rats are sacrificed when the tumor reaches $>10,000$ mm³ or the tumor ulcerates; thus, the tumors in our experiments were initially much smaller than those in the investigation of Stolz *et al.* (4). The size of the tumor at the beginning of the treatment may account for the difference in the response of the tumors to the different radionuclides; therefore, a meaningful comparison cannot be made between the efficacy of the ^{90}Y and ^{64}Cu compounds at this time. Moreover, the treatment of the tumor with ^{64}Cu -TETA-Y3-TATE may lead to the selective killing of receptor-rich cells. Theoretically, multiple dose schedules may cause a significant decrease in somatostatin receptor density after repeated administrations. As a consequence, regrowth of CA20948 tumors after treatment is possible because cells with a smaller number or no somatostatin receptors survive during ^{64}Cu -TETA-Y3-TATE treatment. Therefore, because of the longer mean range of the ^{90}Y β^- particle, receptor-negative bystander cells could also be killed. This may also account, in part, for the complete eradication of CA20948 tumors reported by Stolz *et al.* (4).

In the 3×20 mCi dose study reported here, the treated rats gained weight throughout the experiment and at no time presented with any overt physical signs of toxicity, such as lethargy, scruffy coat, $>10\%$ weight loss, or diarrhea. Transient elevation and decrease in certain hematological and enzyme levels were noted, but by day 34 after the first treatment, all levels returned to baseline values. Although not fully comprehensive, these toxicity data are encouraging in that a maximum tolerated dose was not achieved and that larger quantities of radioactivity could be administered safely.

The biodistribution of ^{64}Cu -TETA-Y3-TATE in rats was clearly affected by the mass of peptide injected. A bell-shaped relationship has been reported between mass of ^{111}In -pentetreotide and its uptake in receptor-rich tissues (30). In this investigation, there was maximum uptake in somatostatin receptor-positive tissues at the lowest mass dose (10 ng), with de-

creasing uptakes in these tissues at higher masses. For the specific activities given in this report (1.25–2.5 mCi/ μg), this would convert to 4–16 μg of material being injected in the 10- and 20-mCi treatment doses. This would result in the lowering of uptake of ^{64}Cu -TETA-Y3-TATE into receptor-rich tissues, which would have direct consequence on the regression of the tumor and dosimetry estimations. Using ^{64}Cu -TETA-Y3-TATE labeled at high specific activities may improve its therapeutic efficacy.

On the basis of the estimated absorbed doses from the baboon PET images, a typical injectate of ^{64}Cu -TETA-Y3-TATE for an imaging study will result in a total dose to the kidneys of 1.25 rad/mCi (0.337 mGy/MBq). This appears to be the dose-limiting organ, because the bladder dose measured in a baboon would be reduced >7-fold by a normal voiding scheme. The kidney dose determined from the nonhuman primate is ~3-fold higher than what was determined from rats. Dosimetry data presented for the intestinal tract also showed distinctly different results from the rat and baboon experiments. The different biodistribution between nonhuman primates and rodents is not surprising, given that hepatobiliary and renal clearance of many radiopharmaceuticals vary widely from rodents to mammals (31, 32). The decreased intestinal uptake and increase of renal dose in the baboon is likely to be more representative of human biodistribution.

Although the large discrepancies between rodent and primate biodistributions of radiopharmaceuticals are not surprising, the fact that human absorbed dose estimates of ^{64}Cu -TETA-Y3-TATE based on rat biodistribution data are greatly underestimated compared with doses obtained from baboon PET imaging data are problematic. Previous studies in our group on the dosimetry of ^{64}Cu -labeled monoclonal antibody 1A3-F(ab')₂ showed that absorbed dose estimates from rat biodistribution data overestimated what was found from baboon PET imaging data (33). Preliminary studies of ^{64}Cu -TETA-OC in patients showed that absorbed dose estimates based on rat biodistribution data were not greatly different from actual absorbed doses determined from human PET images.⁴ Because of the distinct differences in the biodistribution of ^{64}Cu -TETA-Y3-TATE in rodents and primates, we will base future dosimetry estimates on radiolabeled somatostatin analogues from primate data prior to human studies.

Although human absorbed doses were not estimated in the radiotherapy studies reported previously using ^{188}Re and ^{90}Y -labeled somatostatin analogues in tumor-bearing mice (28, 34), dosimetry results have been reported in the two of the clinical case studies of ^{111}In -DTPA-octreotide therapy. In the report by Krenning *et al.* (8), the patient received a total of 550 mCi (20.4 GBq) over seven administrations, and the estimated doses to the liver and kidneys were 240 and 500 rad (2.4 and 5.0 Gy), respectively, whereas the estimated dose to the tumor was 1300 rad (13 Gy). Fjälling *et al.* (9) reported doses of 630 rads (6.3 Gy) to the liver (which had liver metastases), 371 rad (3.71 Gy) to the spleen, and 212 rad (2.12 Gy) to the kidney and red marrow. A study by Cremonesi *et al.* (35) used ^{111}In -DOTA-

Y3-OC to estimate the absorbed doses that would be received in a therapy study with ^{90}Y -DOTA-Y3-OC. They reported the average estimated dose that would be given for a ^{90}Y therapy trial where 30 mCi (1.1 GBq) was administered per cycle for three cycles. The estimated absorbed doses due to ^{90}Y -DOTA-Y3-OC were 231 rads (2.31 Gy) to the liver, 2508 rad (25.08 Gy) to the spleen, 1089 rad (10.89 Gy) to the kidney, 9 rad (0.09 Gy) to red marrow, and 3333 rad (33.33 Gy) to the tumor. In the present study reported here, a single dose of 15.5 mCi of ^{64}Cu -TETA-Y3-TATE was administered to tumor-bearing rats, which weighed ~250 g. Extrapolating this to humans would suggest a total dose of about 4000 mCi (148 GBq) of ^{64}Cu -TETA-Y3-TATE for clinical therapy trials. On the basis of the nonhuman primate data, a delivered dose of 4000 mCi (148 GBq) of ^{64}Cu -TETA-Y3-TATE would result in absorbed doses of 5000 rad (50 Gy) to the kidney, 1560 rad (15.60 Gy) to the liver, 1400 rad (14 Gy) to the spleen, and 152 rad (1.52 Gy) to the red marrow. The kidneys are the critical organ in this study, and a reduction in kidney absorbed dose would be necessary. Methods have been used to decrease the uptake of radiolabeled proteins and peptides in the kidneys, in particular after the i.v. administration of D-lysine (13).

The absorbed dose to the CA20948 tumor from ^{64}Cu -TETA-Y3-TATE, calculated from the rat biodistribution, was 40.8 rad/mCi (11.0 mGy/MBq), compared with 30.9 rad/mCi (8.4 mGy/MBq) from ^{64}Cu -TETA-OC (7). It is important to note that this is the dose to the rat tumor and not an estimated dose to human tumors. By simple calculation, this represents a dose of 6120 mGy (612 rad) for ^{64}Cu -TETA-Y3-TATE to the CA20948 tumor in the single-dose experiment (1×15.5 mCi).

The data presented here clearly demonstrate that, for targeted radiotherapy with ^{64}Cu -TETA-Y3-TATE, the use of a multiple dose schedule is superior to single injections in terms of efficacy and toxicity. However, the effect of multiple doses on the tumor uptake of ^{64}Cu -TETA-Y3-TATE must be considered for each consecutive injection. Preliminary studies suggest that uptake of ^{64}Cu -TETA-Y3-TATE in receptor-positive organs and the CA20948 tumor decreases for subsequent identical injections given at 48-h intervals (36). In this study, it was shown that tissue uptake was less affected with the longer intervals between administrations (72 h) and suggests that longer dose fractionation protocols may be superior in therapeutic efficacy than 48-h treatment regimens. Future studies in tumor-bearing rats will include the use of MicroPET imaging (Concorde Microsystems, Knoxville, TN) to determine the optimal time interval for multiple dose regimens. PET imaging will enable the calculation of biodistribution data (*i.e.*, dosimetry measurements) and the determination of therapeutic efficacy simultaneously.

In conclusion, ^{64}Cu -TETA-Y3-TATE was effective in causing tumor regression of CA20948 tumors in rats. A multiple dose regimen of ^{64}Cu -TETA-Y3-TATE temporarily eradicated CA20948 tumors, without lethal toxicity to the animal. It is clear that optimization of the radiotherapeutic multiple dose regimen is necessary to improve upon the results presented in this report. The results reported here also showed significant discrepancies between absorbed dose estimates obtained from rat and baboon biodistribution. These data suggest that ^{64}Cu -TETA-Y3-TATE may not be optimal agent for targeted radiotherapy but does,

⁴ C. J. Anderson *et al.*, manuscript in preparation.

however, confirm that other ^{64}Cu -labeled somatostatin analogues warrant continued consideration as agents for targeted radiotherapy.

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