



## In Vitro and In Vivo Evaluation of $^{64}\text{Cu}$ -TETA-Tyr<sup>3</sup>-Octreotate. A New Somatostatin Analog with Improved Target Tissue Uptake

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**ABSTRACT.** Radiolabeled somatostatin analogs have demonstrated potential as cancer therapeutic agents. Many of these agents are based on the analog octreotide (OC). Recently it has been shown that substitution of a tyrosine for phenylalanine in the 3-position and changing the C-terminus from an alcohol to an acid improves the targeting of somatostatin-rich tissues. The compound, 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid-Tyr<sup>3</sup>-octreotate (TETA-Y3-TATE), was synthesized and radiolabeled with  $^{64}\text{Cu}$ . The receptor binding properties of  $^{64}\text{Cu}$ -TETA-Y3-TATE showed an estimated  $K_d$  value of 549 pM in somatostatin receptor-positive CA20948 tissue membrane. High tumor uptake was observed in two animal tumor models. Tumor uptakes of 2.37 %ID/g in CA20948 tumor-bearing rats and 21.60 %ID/g in AR42J tumor-bearing SCID mice were observed at 1 h, compared with 1.09 %ID/g and 11.24 %ID/g for  $^{64}\text{Cu}$ -TETA-OC. Higher uptake in other somatostatin-receptor rich tissues was also observed, compared with  $^{64}\text{Cu}$ -TETA-OC. Positron emission tomography (PET) imaging with  $^{64}\text{Cu}$ -TETA-Y3-TATE in a baboon showed significant uptake in the pituitary and adrenals, and clearance through the kidneys.  $^{64}\text{Cu}$ -TETA-Y3-TATE, a new OC analog for binding somatostatin receptors, demonstrated significantly greater uptake in somatostatin-rich tissues in two tumor-bearing animal models, and demonstrated great potential as a radiopharmaceutical for imaging and therapy of somatostatin receptor-positive tissues. NUCL MED BIOL 26;3:267–273, 1999. © 1999 Elsevier Science Inc. All rights reserved.

**KEY WORDS.** Copper radionuclides, Somatostatin, Octreotide, Tyr<sup>3</sup>-octreotate, PET

### INTRODUCTION

The peptide hormone somatostatin is a 14-amino-acid cyclic peptide that is involved in the regulation of several organ systems in the human body. Receptors for this peptide are expressed in those organ systems and are also up-regulated in certain types of tumors (23). Significant attention has been focused on the therapeutic potential of somatostatin on tumors of the neuroendocrine system (22, 24), lung (26), and the central nervous system (CNS) (25). Somatostatin has a short biological half-life due to enzymatic degradation, but octreotide (OC), a cyclic 8-amino-acid peptide analog of somatostatin, is more stable biologically than somatostatin. For diagnostic imaging, octreotide has been labeled with  $^{111}\text{In}$  (Pentetreotide) (3),  $^{18}\text{F}$  (31),  $^{99\text{m}}\text{Tc}$  (18),  $^{64}\text{Cu}$  (2),  $^{68}\text{Ga}$  (29),  $^{86}\text{Y}$  (31), and  $^{123}\text{I}$  (4, 6, 14).  $^{111}\text{In}$ -diethylenetriaminepentaacetic acid-octreotide ( $^{111}\text{In}$ -DTPA-OC) is approved for routine clinical use as

a diagnostic agent for neuroendocrine cancer in the US and Europe (3, 4).

The use of radiolabeled somatostatin analogs for targeted radiotherapy is becoming more widespread.  $^{188}\text{Re}$ -RC-160 (33),  $^{161}\text{Tb}$ -DTPA-OC (12),  $^{90}\text{Y}$ -1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-Tyr<sup>3</sup>-OC ( $^{90}\text{Y}$ -DOTA-Y3-OC) (21, 30),  $^{90}\text{Y}$ -DTPA-OC (28), and  $^{64}\text{Cu}$ -1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid-OC ( $^{64}\text{Cu}$ -TETA-OC) (1) have all been suggested as candidates. These agents are based on the OC analog.  $^{64}\text{Cu}$  ( $t_{1/2} = 12.8$  h,  $\beta^+ = 0.655$  [19.3%],  $\beta^- = 0.573$  [39.6%]) has emissions suitable for positron emission tomography (PET) imaging and radiotherapy and its attachment to small molecules and biomolecules for use in nuclear medicine has been reviewed (5). Large quantities of this isotope can now be produced in high specific activity as needed using a biomedical cyclotron (19).  $^{64}\text{Cu}$ -TETA-OC is in clinical trials for the PET imaging of somatostatin receptor-positive tumors (9).

Recently it has been shown that substitution of a tyrosine for phenylalanine in the 3-position and changing the C-terminus from an alcohol to an acid improves the targeting to somatostatin-rich tissues (10, 11). Herein we report the *in vitro* and *in vivo* evaluation of a new somatostatin analog,  $^{64}\text{Cu}$ -TETA-Y3-TATE. Y3-TATE differs from OC in that Tyr replaces Phe in the 3-position and the C-terminal threonine is an acid rather than an alcohol (Fig. 1). We demonstrate binding of  $^{64}\text{Cu}$ -TETA-Y3-TATE to the somatostatin receptor both *in vitro* and *in vivo* in three animal models.

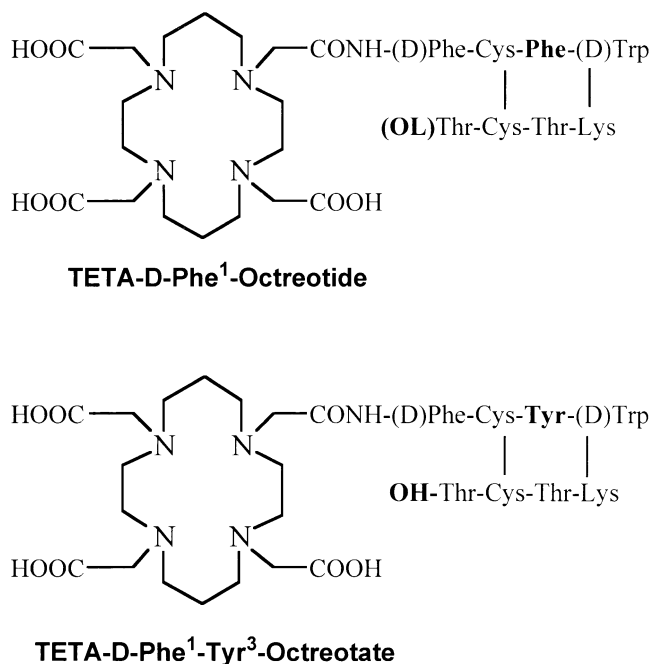
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Abbreviations: DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; TETA, 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid; Y3, Tyr<sup>3</sup>; OC, octreotide; TATE, octreotate.

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**FIG. 1. Schematic representations of TETA-D-Phe<sup>1</sup>-octreotide (TETA-OC) and TETA-D-Phe<sup>1</sup>-Tyr<sup>3</sup>-Octreotide (TETA-Y3-TATE) used in this study.**

## MATERIALS AND METHODS

<sup>64</sup>Cu was produced on a biomedical cyclotron at the Washington University School of Medicine as reported previously (19). All chemicals unless otherwise stated were purchased from Aldrich (Milwaukee, WI). All solutions were prepared using distilled deionized water (Milli-Q; >18 MΩ resistivity). Thin layer chromatography (TLC) was performed using Whatman MKC<sub>18</sub>F reversed-phase TLC plates with 70:30 methanol:5% ammonium acetate as the mobile phase. Radio-TLC was carried out on a BIOSCAN System 200 Imaging scanner (Washington, DC). Radioactive samples were counted on a Beckman 8000 gamma counter (Irvine, CA). Female SCID-Fox Chase CB-17 mice (29–35 days old) were purchased from Charles River Laboratories (Wilmington, MA). Adult male Lewis rats (230–290 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN). The rat pancreatic tumor line CA20948 (16) was obtained from the Tumor Bank at Biomeasure (Hopkinton, MA) and the AR42J tumor line was obtained from American Type Culture Collection (ATCC, Rockville, MD). Both cell lines were maintained by serial passage in animals.

Solid phase peptide synthesis (SPPS) was achieved using an Applied Biosystems Model 432A “Synergy” Peptide synthesizer employing the 9-fluorenylmethoxycarbonyl (Fmoc) strategy. The instrument protocol required 25 mmol of subsequent Fmoc-protected amino acids activated by a combination of *N*-hydroxybenzotriazole (HOBt) and 2-(1-*H* Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). The Fmoc-protected amino acids were purchased commercially unless otherwise stated; the prepacked amino acids were obtained from Perkin-Elmer (Norwalk, CT), whereas those unavailable prepacked such as, the *D*-amino acids and Fmoc-Cys acetamidomethyl (Acm) were supplied by BACHEM Bioscience (King of Prussia, PA) or Novabiochem (San Diego, CA). Tri-*tert*-butyl TETA was synthesized by a modification of the published procedure (20). Molecular weight determination was

accomplished by mass spectrometry (MS) operating in the electro-spray mode (ESI).

## Synthesis of Y3-TATE and TETA-Y3-TATE

The linear, protected Y3-TATE, Fmoc-*D*-Phe-Cys(Acm)-Tyr(OtBu)-*D*-Trp(Boc)-Thr(OtBu)-Cys(Acm)-Thr(OtBu), was prepared by standard automated SPPS. At the end of the synthesis, thallium(III) trifluoroacetate (75 μmol) was used to remove the cysteines’ Acm-protecting groups concomitant with disulfide formation to generate the cyclic peptide. This resin was then put back into the synthesizer for the conjugation of TETA to the *N*-terminus by the introduction of the tri-*tert*-butyl TETA derivative described above. It was necessary to incorporate the TETA ligand after the thallium-mediated cyclization to prevent chelation of the thallium. The resulting peptide was cleaved from the resin and deprotected with trifluoroacetic acid:thioanisole:phenol:water (85:5:5:5) for 8–10 h. The peptide was then precipitated by the addition of 10 mL of *tert*-butylmethyl ether, and the peptide-resin mixture was washed with 4 × 10 mL of *tert*-butylmethyl ether. Acetonitrile:water (2:3) was added to dissolve the peptide; the spent resin was removed by filtration. The solution containing the crude peptide was lyophilized prior to purification by high performance liquid chromatography (HPLC). Final purification was accomplished by C-18 reversed-phase chromatography (Solvent A: 0.1% trifluoroacetic acid [TFA]/water, Solvent B: 0.1% TFA/10% H<sub>2</sub>O/acetonitrile: Gradient: 90% A/10% B to 30% A/70% B in 40 min; detection mode: ultraviolet [UV] at 230 nm). Fractions were analyzed by an analytical HPLC (detection mode: UV at 214 nm) prior to final lyophilization.

## Preparation of <sup>64</sup>Cu-TETA-OC and <sup>64</sup>Cu-TETA-Y3-TATE

The radiolabeling of <sup>64</sup>Cu-TETA-OC and <sup>64</sup>Cu-TETA-Y3-TATE was based on methods reported previously for the production of <sup>64</sup>Cu-TETA-OC (2). Briefly, 1–5 mCi (37–185 MBq) <sup>64</sup>Cu in 0.1 M NH<sub>4</sub>OAc (pH 5.5) were added to 1–10 μg TETA-OC or TETA-Y3-TATE in 0.1 M NH<sub>4</sub>OAc buffer at pH 5.5. Gentic acid (1 mg/mL) was added to the labeling mixture to counteract the effects of radiolysis. The solution was incubated for 1 h at 37°C. The radiolabeled compound was purified by SepPak procedures (elution in 100% ethanol) and radiochemical purity was determined by Radio-TLC.

## Receptor Binding Assays

The receptor binding assays were performed with the cell line CA20948 harvested from euthanized rats, with <sup>nat</sup>Cu-TETA-Y3-TATE as a competing ligand. The generation of the dissociation constant (*K<sub>d</sub>*), *IC*<sub>50</sub>, and the receptor concentration (*B<sub>max</sub>*) were based on methods reported previously (1). Experiments were conducted using the Millipore MultiScreen system (Bedford, MA). For data analysis, the GraFit program (Erithacus Software, UK) and LIGAND were used.

## Animal Biodistribution Studies

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.

Three rodent biodistributions were performed on two tumor-bearing animal models. The tumors were maintained by serial

**TABLE 1. Biodistribution at 1, 4, and 24 h of  $^{64}\text{Cu}$ -TETA-Y3-TATE,  $^{64}\text{Cu}$ -TETA-Y3-TATE with Coinjection of Blocking Dose of Y3-TATE, and  $^{64}\text{Cu}$ -TETA-OC at 1 h in Lewis Rats Bearing CA20948 Pancreatic Tumor**

	1 h $^{64}\text{Cu}$ -TETA-OC	1 h $^{64}\text{Cu}$ -TETA-Y3-TATE	1 h $^{64}\text{Cu}$ -TETA-Y3-TATE with Y3-TATE block	4 h $^{64}\text{Cu}$ -TETA-Y3-TATE	24 h $^{64}\text{Cu}$ -TETA-Y3-TATE
Blood	0.16 ± 0.04	0.09 ± 0.01	0.15 ± 0.02	0.08 ± 0.02	0.10 ± 0.02
Lung	0.14 ± 0.02	0.17 ± 0.03	0.12 ± 0.02	0.12 ± 0.02	0.09 ± 0.01
Liver	0.16 ± 0.02	0.21 ± 0.03	0.20 ± 0.03	0.30 ± 0.05	0.33 ± 0.03
Spleen	0.06 ± 0.01	0.08 ± 0.03	0.07 ± 0.02	0.05 ± 0.01	0.09 ± 0.01
Kidney	2.02 ± 0.24	1.54 ± 0.16	2.27 ± 0.35	1.60 ± 0.18	1.19 ± 0.16
Muscle	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.16 ± 0.01	0.02 ± 0.01
Fat	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Heart	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Pituitary	0.85 ± 0.14	6.47 ± 1.77	0.39 ± 0.30	6.51 ± 0.43	2.74 ± 1.68
Bone	0.11 ± 0.01	0.52 ± 0.03	0.09 ± 0.02	0.38 ± 0.07	0.11 ± 0.02
Adrenals	1.26 ± 0.24	9.07 ± 1.24	0.17 ± 0.02	6.38 ± 0.96	1.68 ± 0.19
Pancreas	1.20 ± 0.30	9.35 ± 1.66	0.15 ± 0.02	5.67 ± 1.29	0.63 ± 0.10
Tumor	1.09 ± 0.22	2.37 ± 0.44	0.22 ± 0.02	2.22 ± 0.26	0.55 ± 0.10

%ID/g ± SD, n = 5.

passage in animals and each tumor was initiated by the implantation of 1 mm<sup>3</sup> piece of freshly excised tumor. The somatostatin receptor-positive rat pancreatic tumor, CA20948, was implanted subcutaneously into the nape of the neck of male Lewis rats (230–290 g) and the tumors were allowed to grow until approx. 4 g in size. The somatostatin receptor-positive pancreatic tumor, AR42J, was implanted subcutaneously, bilaterally, into the flanks of female SCID mice (29–35 days old) and the tumors were allowed to grow until 0.1–0.9 g in size.

$^{64}\text{Cu}$ -TETA-Y3-TATE (5.4  $\mu\text{Ci}$ , 5 ng) was injected intravenously (IV) via the tail vein into CA20948 tumor-bearing Lewis rats. Animals were killed at 1, 4, and 24 h postinjection (n = 5). An additional group of animals received  $^{64}\text{Cu}$ -TETA-OC in the same manner and were sacrificed at 1 h postinjection. Another group of rats were co-injected with 150  $\mu\text{g}$  Y3-TATE and  $^{64}\text{Cu}$ -TETA-Y3-TATE and sacrificed at 1 h postinjection. The Lewis rats for the 24-h time point were placed in metabolism cages immediately after injection until sacrifice. Urine and feces were collected at various times from 1 to 24 h, and counted on a gamma counter, and the percent injected dose excreted was determined.

$^{64}\text{Cu}$ -TETA-Y3-TATE (5.4  $\mu\text{Ci}$ , 2 ng) was injected IV via the tail vein of AR42J-bearing SCID mice. Animals were sacrificed at 1, 4, and 23 h postinjection (n = 5). An additional group of animals received  $^{64}\text{Cu}$ -TETA-OC in the same manner and were sacrificed at 1 h postinjection.

For both tumor-bearing animal models, the tumor and selected organs and tissues (blood, lung, liver, spleen, kidney, muscle, fat, heart, brain, pituitary, bone, adrenals, pancreas, stomach, small intestine, upper large intestine, and lower large intestine) were removed, weighed, and the activity counted on a gamma counter. The percent injected dose per gram (%ID/g) and percent injected dose per organ (%ID/organ) for each tissue were calculated.

To determine if the observed difference between compounds was significant, a Student's *t* test was performed and a 95% confidence level assumed, with *p* < 0.05 being significantly different.

### Primate Imaging Study

A male baboon (27 kg) was imaged 1 h following IV administration of 3.5 mCi (130 MBq) of  $^{64}\text{Cu}$ -TETA-Y3-TATE on a Siemens/CTI

ECAT EXACT 921 system (CTI PET Systems, Knoxville, TN). Imaging proceeded, from the pelvis up to and including the brain in four steps consisting of an 8-min static emission scan followed by a 2-min, postinjection transmission scan. Transmission scans were subsequently processed with a segmentation algorithm to yield noiseless attenuation maps, free of the typical postinjection artifacts (32).

Tomographic images were reconstructed using an accelerated implementation of estimation maximization (EM) algorithm. This reconstruction technique, known as OSEM, provides improved signal-to-noise with a slightly longer construction time. The four-image segments were combined and then smoothed to produce the projected whole-body images.

## RESULTS

### Preparation of TETA-Y3-TATE and $^{64}\text{Cu}$ -TETA-Y3-TATE

HPLC showed TETA-Y3-TATE had a retention time of 11.33 min and the product was isolated with a purity of greater than 98%. ESI-MS confirmed a product of molecular weight 1049.5 (M+1).  $^{64}\text{Cu}$ -TETA-Y3-TATE was obtained in >98% radiochemical purity, as analyzed by radio-TLC, in specific activities ranging from 0.5 to 2.5 mCi/ $\mu\text{g}$  (18–65 MBq/ $\mu\text{g}$ ).

### Receptor Binding Assays

In the receptor binding studies,  $^{64}\text{Cu}$ -TETA-Y3-TATE was observed to have an estimated  $K_d$  of 549 pM. The  $\text{IC}_{50}$  vs.  $^{nat}\text{Cu}$ -TETA-Y3-TATE was determined to be  $0.25 \pm 0.05$  nM. The receptor density in CA20948 membranes ( $B_{\text{max}}$ ) was found to be 122.6 fmol/mg protein (15% CV). The  $\text{IC}_{50}$  value for Cu-TETA-OC was reported previously to be  $0.498 \pm 0.039$  nM, with an estimated  $K_d$  of 617 pM (1).

### Animal Biodistribution Studies

The biodistribution data for  $^{64}\text{Cu}$ -TETA-Y3-TATE in Lewis rats bearing CA20948 tumors are shown in Table 1.  $^{64}\text{Cu}$ -TETA-Y3-TATE was extracted rapidly from the blood in the tumor-bearing

**TABLE 2. Biodistribution at 1, 4, and 23 h of <sup>64</sup>Cu-TETA-Y3-TATE, <sup>64</sup>Cu-TETA-OC at 1 h in SCID Mice Bearing AR42J Pancreatic Tumor**

	1 h <sup>64</sup> Cu-TETA-OC	1 h <sup>64</sup> Cu-TETA-Y3-TATE	4 h <sup>64</sup> Cu-TETA-Y3-TATE	23 h <sup>64</sup> Cu-TETA-Y3-TATE
Blood	0.39 ± 0.03	1.15 ± 0.23	0.75 ± 0.24	0.93 ± 0.32
Lung	7.42 ± 0.20	28.13 ± 4.12	20.16 ± 1.95	6.01 ± 1.04
Liver	2.29 ± 0.23	7.07 ± 1.50	6.90 ± 1.46	5.79 ± 1.32
Spleen	1.16 ± 0.29	3.25 ± 0.44	3.03 ± 0.48	2.61 ± 0.55
Kidney	6.92 ± 0.40	8.55 ± 2.49	4.17 ± 0.66	2.86 ± 0.61
Muscle	0.18 ± 0.01	0.36 ± 0.06	0.30 ± 0.08	0.27 ± 0.07
Fat	1.19 ± 1.68	0.76 ± 0.37	0.29 ± 0.17	0.67 ± 0.42
Heart	0.51 ± 0.07	1.88 ± 0.30	1.69 ± 0.29	1.71 ± 0.35
Bone	1.26 ± 0.25	5.05 ± 0.86	2.46 ± 0.19	0.72 ± 0.18
Adrenals	1.62 ± 0.82	5.16 ± 1.39	3.08 ± 0.95	3.35 ± 1.02
Pancreas	1.55 ± 0.10	29.41 ± 7.76	5.38 ± 1.38	1.58 ± 0.11
Tumor	11.24 ± 2.84	21.60 ± 6.03	14.53 ± 4.59	5.71 ± 0.72

%ID/g ± SD, n = 5.

Lewis rats after 1 h and showed similar biodistribution in the non-somatostatin receptor-positive organs similar to <sup>64</sup>Cu-TETA-OC. In the somatostatin receptor-positive tissues (adrenals, pancreas, pituitary, and tumor), <sup>64</sup>Cu-TETA-Y3-TATE exhibited higher uptake compared with <sup>64</sup>Cu-TETA-OC. The adrenals (9.07 ± 1.24 %ID/g), pancreas (9.35 ± 1.66 %ID/g), and pituitary (6.47 ± 1.77 %ID/g) showed significantly higher uptake at 1 h for <sup>64</sup>Cu-TETA-Y3-TATE compared with <sup>64</sup>Cu-TETA-OC (adrenals: 1.26 ± 0.24 %ID/g; pancreas: 1.20 ± 0.30 %ID/g; pituitary: 0.85 ± 0.14) (*p* < 0.001). In the tumor, the uptake of <sup>64</sup>Cu-TETA-Y3-TATE at 1 h (2.37 ± 0.44 ID/g) was more than twice that observed for <sup>64</sup>Cu-TETA-OC (1.09 ± 0.22 % ID/g). More than 90% of the activity in somatostatin-rich tissues was blocked with co-injection of 150 μg Y3-TATE, demonstrating specific uptake.

The same trends were observed in the AR42J tumor-bearing SCID mice (Table 2). Most notably, the tumor uptake of <sup>64</sup>Cu-TETA-Y3-TATE (21.60 ± 6.03 %ID/g) was again approximately twice that of <sup>64</sup>Cu-TETA-OC (11.24 ± 2.84 %ID/g).

The uptake of <sup>64</sup>Cu-TETA-Y3-TATE in bone at 1 h in CA20948-bearing rats (0.52 ± 0.03 %ID/g) and in SCID mice (5.05 ± 0.86 %ID/g) was significantly higher than that of <sup>64</sup>Cu-TETA-OC (0.11 ± 0.01 %ID/g and 1.26 ± 0.25 %ID/g for rats and mice, respectively, *p* < 0.001), and decreased in the competitor ligand experiment (0.09 ± 0.02 %ID/g for the Y3-TATE block in rats). Bone marrow was harvested from bone mineral in tumor-bearing rats, and was shown to contain significantly lower levels of radioactivity (0.13 ± 0.08 %ID/g in marrow vs. 0.57 ± 0.11 %ID/g for bone surface, *p* < 0.001), suggesting that somatostatin receptors were present on the bone surfaces.

In SCID mice, high lung uptake was observed at 1 h. This activity was retained over 4 h (20.16 %ID/g) decreasing to 6.01

%ID/g after 23 h. The rat model used in this study did not show high lung uptake (0.17 %ID/g lung at 1 h), and no significant blocking was observed in the competitor ligand experiments in the rat lung.

Selected organ-to-tissue ratios are given in Table 3. The data show the highest tumor: blood ratios at 1 or 4 h postinjection in both tumor-bearing rodent models, due to fairly stable blood concentration, but significant clearance from the tumor between 4 and 24 h.

Excretion data from the CA20948-bearing Lewis rats are presented in Table 4. <sup>64</sup>Cu-TETA-Y3-TATE was cleared primarily through the kidneys (56% over 24 h), however, significant fecal excretion was seen at 24 h (20.0 %ID/organ).

Figure 2 shows a PET image of a male baboon after injection of 3.6 mCi of <sup>64</sup>Cu-TETA-Y3-TATE. The area of highest activity was the bladder. The pituitary, kidneys, liver, and the adrenals were clearly visualized. The uptake in the lung and bone was not observed in the primate image.

## DISCUSSION

<sup>64</sup>Cu-TETA-OC is currently being evaluated in humans at Washington University School of Medicine for the PET imaging of somatostatin receptor-positive tumors (9). The preliminary results in humans are promising because more tumors have been seen with <sup>64</sup>Cu-TETA-OC and PET than with <sup>111</sup>In-Pentetreotide and gamma scintigraphy. Some disadvantages of <sup>64</sup>Cu-TETA-OC include rapid clearance from the tumor and less than optimal blood clearance. Our aim is to develop an improved agent for PET imaging of somatostatin receptors. <sup>64</sup>Cu-TETA-Y3-TATE, a new octreotide analog for binding somatostatin receptors, demonstrated

**TABLE 3. Selected Organ-to-Tissue Ratios (%ID/g) of <sup>64</sup>Cu-TETA-Y3-TATE in Tumor-Bearing Rats and Mice**

	Lewis rats bearing CA20948 tumor			SCID mice bearing AR42J tumor		
	1 h	4 h	24 h	1 h	4 h	23 h
Tumor: blood	27.7 ± 5.6	28.1 ± 6.7	6.3 ± 2.2	20.0 ± 7.1	20.7 ± 9.4	7.0 ± 1.8
Tumor: liver	11.9 ± 1.6	8.0 ± 1.9	1.7 ± 0.8	3.1 ± 0.7	2.2 ± 0.8	1.0 ± 0.1
Tumor: kidney	1.6 ± 0.3	1.4 ± 0.1	0.5 ± 0.1	2.8 ± 0.6	3.5 ± 1.1	2.1 ± 0.2
Tumor: muscle	81.9 ± 25.3	139.7 ± 20.5	33.6 ± 5.8	64.4 ± 23.5	50.3 ± 20.4	22.2 ± 2.9

**TABLE 4. Excretion of  $^{64}\text{Cu}$ -TETA-Y3-TATE in CA20948 Tumor-Bearing Lewis Rats**

	4 h	14.5 h	24 h	Total over 24 h
Urine	24.05 ± 18.48	31.90 ± 23.57	0.81 ± 0.34	56.75 ± 5.66
Feces	—	16.61 ± 3.08	3.43 ± 0.68	20.04 ± 2.90

All data are given as % injected dose ± SD.

significantly greater uptake in somatostatin-rich tissues in two tumor-bearing animal models, and demonstrated great potential as a radiopharmaceutical for imaging and therapy of somatostatin receptor-positive tissues.

The changes between  $^{64}\text{Cu}$ -TETA-OC and  $^{64}\text{Cu}$ -TETA-Y3-TATE are substituting Tyr for Phe in the 3-position and a C-terminal carboxylic acid for a C-terminal alcohol. The synthesis of TATE derivatives, with the C-terminal acid vs. the C-terminal alcohol, is significantly easier compared with that of OC analogues, because the TATE agents are easily cleaved from standard resins and are produced in higher yields (13).

In the receptor binding studies,  $^{64}\text{Cu}$ -TETA-Y3-TATE was observed to have an estimated  $K_d$  of 549 pM, which suggests strong binding to somatostatin receptors *in vitro*. The receptor affinity of  $^{64}\text{Cu}$ -TETA-Y3-TATE is also shown *in vivo* in somatostatin receptor-positive tissues such as pancreas, pituitary, and adrenal gland, with significant washout from these tissues over 24 h. In rats co-injected with a blocking dose of Y3-TATE, the uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE in these tissues was reduced dramatically. This modulation of uptake was also observed in tissues not normally considered to have a high concentration of somatostatin receptors, e.g., bone. The uptake in the pancreas, pituitary, and adrenal glands as well as the bone and lung was markedly higher than that observed with  $^{64}\text{Cu}$ -TETA-OC at 1 h in both animal models. These differences in uptake may suggest: (a) differences in the biological kinetics of  $^{64}\text{Cu}$ -TETA-Y3-TATE (e.g., different off-rates, internalization rates); (b) the ability of  $^{64}\text{Cu}$ -TETA-Y3-TATE to bind to different somatostatin receptor subtypes; and (c)  $^{64}\text{Cu}$ -TETA-Y3-TATE and  $^{64}\text{Cu}$ -TETA-OC may have different affinities for the same receptor subtype. The suggestion that the two  $^{64}\text{Cu}$ -radiolabeled peptides may have different affinities for the same receptor

subtype is confirmed by the difference in the relative  $K_d$  values reported for  $^{64}\text{Cu}$ -TETA-Y3-TATE and  $^{64}\text{Cu}$ -TETA-OC (549 nM vs. 617 pM, respectively).

In both rats and mice, there was specific uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE in the bone that was not observed with  $^{64}\text{Cu}$ -TETA-OC. Somatostatin receptors in bone cells are believed to be involved in the direct regulation of bone formation (7, 17). It has been shown that injection of radiiodinated Y3-OC ( $^{125}\text{I}$ ]SDZ 204-090) after injection of unlabelled compound reduced bone uptake in neonatal rats (17). The study showed that high populations of receptors are accessible to circulating somatostatin during bone development. The data presented here show a particularly high uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE in the bone of the SCID mice bearing the AR42J tumor (5.05 %ID/g at 1 h). The CA20948-bearing Lewis rats had a bone uptake of 0.52 %ID/g. The Lewis rats used in the study were mature, whereas the SCID mice used were younger and therefore may have had a greater amount of forming bone. Studies described here and by others (7, 17) suggest that the younger mice will likely have a higher concentration of somatostatin receptors in bone.

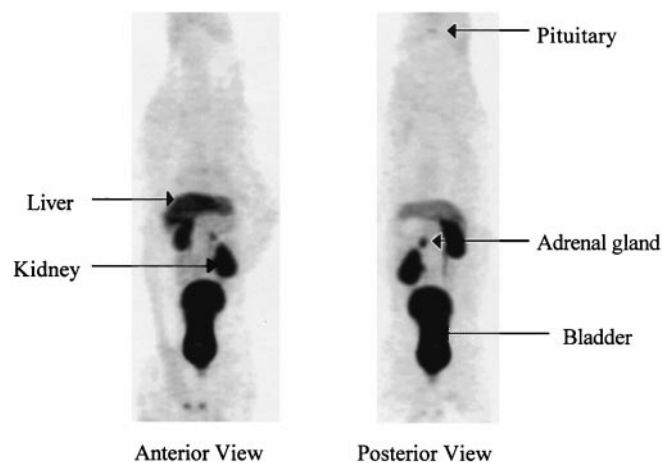
In mice there was also receptor-mediated uptake in the lungs. Bruns and co-workers have shown somatostatin receptors in lung (8, 27). They showed that  $^{125}\text{I}$ ]SRIF-28 bound with high affinity to lung tissue could be displaced by unlabeled somatostatin analogs. The somatostatin receptor subtype SSTR4 was reported to be predominant in rat, mouse, and human lung tissue, although the functional role of this receptor remains to be elucidated.

$^{64}\text{Cu}$ -TETA-Y3-TATE demonstrated significant receptor-mediated uptake in both the CA20948 and the AR42J pancreatic tumor systems. This uptake and the higher tumor-to-nontarget organ ratios over a 24-h period are very encouraging for possible clinical utility. Further, on comparison with the tumor uptake of  $^{64}\text{Cu}$ -TETA-OC, a two-fold increase in uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE was observed in the CA20948 and the AR42J tumors.

A recent report has compared the membrane receptor binding properties of  $^{111}\text{In}$ -DTPA-OC,  $^{111}\text{In}$ -DTPA-Y3-OC, and  $^{111}\text{In}$ -DTPA-Y3-TATE and their biodistribution in Lewis rats bearing the CA20948 tumor (11). The Y3 derivatives displayed higher receptor-positive tissue uptake than  $^{111}\text{In}$ -DTPA-OC. Furthermore,  $^{111}\text{In}$ -DTPA-Y3-TATE showed the highest uptake in all target organs. In the current study,  $^{64}\text{Cu}$ -TETA-Y3-TATE uptake at 1 h (2.37 %ID/g) in the CA20948 tumor was comparable to the other agents reported in the literature. This value decreased to 0.55 %ID/g over 24 h. With  $^{111}\text{In}$ -DTPA-OC, at 1 h, 0.57 %ID/g was observed in the CA20948 tumor with a value of 0.32 %ID/g after 24 h (2). With  $^{64}\text{Cu}$ -TETA-OC at 1 h, 1.09 %ID/g is observed in the CA20948 tumor, with a value of 0.24 %ID/g after 24 h (2).

$^{64}\text{Cu}$ -TETA-Y3-TATE is cleared primarily through the kidney (56% of the activity excreted in the urine over 24 h), but more slowly than  $^{64}\text{Cu}$ -TETA-OC (73% in the urine over 24 h) (2).  $^{64}\text{Cu}$ -TETA-Y3-TATE was likely retained in the animals due to increased tumor and somatostatin receptor-positive tissue uptake. Renal clearance permits reducing the absorbed dose to the patient and improving imaging resolution by reducing background. Kidney uptake has been shown to be significantly reduced by the co-administration of D-lysine with radiolabeled octreotide derivatives without affecting the uptake of tracer into receptor-positive tissues (10).

Posterior and anterior baboon images are shown in Figure 2. Notable points include the clear visualization of the adrenals and pituitary. In patients imaged with  $^{64}\text{Cu}$ -TETA-OC and PET, the adrenals have been observed, but these organs are not generally



**FIG. 2. Positron emission tomography (PET) image of the head and torso of a 27-kg baboon injected with  $^{64}\text{Cu}$ -TETA-Y3-TATE and scanned 1 h postinjection.**

observed with  $^{111}\text{In}$ -DTPA-OC (9). However, the pituitary has not been visualized with either agent in the ongoing clinical study. From the posterior view of the baboon image, the pituitary gland is visible at the base of the skull. The baboon image shows no significant bone uptake, suggesting that bone uptake in mature humans will not likely be a problem.

It is difficult to state at this time whether the increased uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE compared with  $^{64}\text{Cu}$ -TETA-OC in somatostatin-rich tissues *in vivo* was due to the C-terminal carboxylate or the substitution of Tyr for Phe at the 3-position. De Jong *et al.* (11) showed with  $^{111}\text{In}$ -radiolabeled derivatives that the substitution of Tyr for Phe produced increased uptake in receptor-positive tissues, although the compound that displayed the highest increase also contained the C-terminal carboxylate moiety. An investigation involving other octreotide/octreotate analogues is underway to determine which structural properties are responsible for the increased uptake in receptor-rich tissues and the uptake in the lung and bone.

## CONCLUSION

$^{64}\text{Cu}$ -TETA-Y3-TATE demonstrated high capacity for binding to somatostatin receptors both *in vivo* and *in vitro*.  $^{64}\text{Cu}$ -TETA-Y3-TATE demonstrated good clearance from blood in the rat model and uptake in the tumor that was twice that of  $^{64}\text{Cu}$ -TETA-OC. Also, tumor:nontarget organ ratios are higher for  $^{64}\text{Cu}$ -TETA-Y3-TATE. Its clearance properties are similar, if not superior, to that of  $^{64}\text{Cu}$ -TETA-OC. The increased uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE in target organs such as the adrenals, bone, and pancreas, compared with  $^{64}\text{Cu}$ -TETA-OC, may have consequences for the whole body dose for patients in clinical imaging protocols. We are currently evaluating the normal organ absorbed doses of  $^{64}\text{Cu}$ -TETA-Y3-TATE for its possible use in PET imaging or as a therapeutic agent for somatostatin receptor-positive tumors in humans (15).

The significantly higher uptake of  $^{64}\text{Cu}$ -TETA-Y3-TATE in somatostatin-rich tissues over  $^{64}\text{Cu}$ -TETA-OC offers the possibility of a new diagnostic PET radiopharmaceutical for the diagnosis of somatostatin receptor-positive cancer.

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