Gallium-67/Gallium-68-[DFO]-Octreotide—A Potential Radiopharmaceutical for PET Imaging of Somatostatin Receptor-Positive Tumors: Synthesis and Radiolabeling In Vitro and Preliminary In Vivo Studies

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When labeled with gamma-emitting radionuclides, somatostatin analogs have the potential to localize somatostatin receptor-positive tumors using gamma camera scintigraphy. We present a somatostatin analog, [DFO]-octreotide (SDZ 216-927), that comprises desferrioxamine B coupled to octreotide via a succinyl linker. This conjugate can be labeled with either ^{67}Ga for gamma scintigraphy or ^{68}Ga for PET imaging. The ^{67}Ga-labeled conjugate is stable in vitro to autoradiolysis over a 24-hr period. **Methods:** Rats bearing a somatostatin receptor-positive pancreatic islet cell tumor were injected with 20 MBq of ^{67}Ga[DFO]-octreotide (33 GBq ^{67}Ga/μmole). **Results:** After 1 hr, the accumulation of ^{67}Ga[DFO]-octreotide was 0.38 ± 0.08 %ID/g and the tumor-to-nontumor ratios for blood, muscle, liver and intestine were 2.5, 7.4, 1.9 and 1.6, respectively. PET studies with ^{68}Ga[DFO]-octreotide recorded a very rapid accumulation at the tumor and a subsequent residence half-life of about 6 hr. **Conclusion:** Gallium-68-[DFO]-octreotide can be used in PET studies to diagnose receptor-positive tumors such as gastroenteropancreatic, small-cell lung and breast tumors.

Key Words: somatostatin receptors; octreotide; gallium-68; gallium-67

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Somatostatin (somatotropin release inhibiting factor, SRIF) is a cyclic tetradecapeptide, initially found in the hypothalamus (*1*), which has an inhibitory effect on growth hormone secretion. SRIF exerts a wide inhibitory effect at a number of sites including the brain, the gut, the pituitary gland, the endocrine and exocrine pancreas and the thyroid (*2,3*). In animal experiments this peptide appears to inhibit the growth of various tumors such as chondrosarcomas, osteosarcomas, breast and prostatic cancers (*4*).

SRIF has a very short plasma half-life and for this reason the biologically active analog octreotide (Sandostatin®, SDZ 201-995) (*5*) was developed. Octreotide is an octapeptide which is longer-acting than the native hormone in vivo because of the resistance to enzyme attack afforded by the incorporation of (D)Phe^1, (D)Trp^4 and Thr(ol) at the sensitive sites of the peptide (*5*). Consequently, it is two-thousand times more effective than somatostatin-14 in the suppression of growth hormone secretion in the rat 1 hr postapplication (*5*).

Somatostatin analogs have been labeled with ^{125}I, ^{123}I and ^{111}In for the detection of somatostatin receptor-positive tumors both in vitro and in vivo (*6–8*). Specifically, [^{123}I-Tyr^3]-octreotide and ^{111}In[DTPA]-octreotide (OctreoScan®111) have been successfully used to visualize somatostatin receptor-positive tumors by gamma camera scintigraphy (*7,8*).

Gallium is important in radiopharmacy because of the ready availability of the isotopes ^{67}Ga and ^{68}Ga. Gallium-67 is a commercial radionuclide used routinely in nuclear medicine for the detection of many types of tumors as well as nonmalignant lesions. When used to label octreotide, this radionuclide could possibly complement ^{111}In in the visualization of somatostatin receptor-positive tumors using either SPECT or conventional gamma camera scintigraphy.

Gallium-68 is a generator-produced PET radionuclide (parent half-life 278 days) which is generally available at most PET centers. The synthesis of a ^{68}Ga receptor-specific PET radiopharmaceutical would enable PET studies to be performed when cyclotron beam time was not available to make the more conventional PET radionuclides. Additionally, radiolabeling by means of a chelator makes use of kit type lyophilized preparations which are currently

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preferred in nuclear medicine and avoids the complex organic chemistry and purification steps which are required for most cyclotron-based PET radiopharmaceuticals. Previous ^{68}Ga-labeled receptor ligands have been based on lipoproteins and albumin (*9,10*).

Several ligand systems, for the labeling of proteins with gallium, have been presented, in particular, desferrioxamine B (DFO) (*11–15*), diethylenetriaminepentaacetic acid (DTPA) (*16–18*) and a N,N′di(2-hydroxybenzyl)ethylenediamine,N,N′-diacetic acid derivative (HBED) (*19*) have been used to label proteins with ^{67}Ga and ^{68}Ga.

Of the three main bifunctional chelating agents used to label proteins and peptides, ^{67}Ga and ^{68}Ga(DTPA) have been shown to be rather unstable against competition from transferrin both in vitro (9% lost per day in human serum) and in vivo (6% lost in 3.5 hr in rhesus monkeys) (*16*). Similarly, the only data available for the stability of a ^{67}Ga(HBED) derivative (*19*) shows a loss of over 20% within 1 day. In comparison, the ^{67}Ga(DFO) complex has been shown to be stable in human serum for 2 days with no loss of $^{67}Ga^{3+}$ (*11*). This data demonstrates a trend by which a bulky ligand (DFO) is relatively stable as opposed to a smaller open ligand (DTPA). A similar trend has been demonstrated for the removal of Fe(III) from transferrin (*20,21*).

The aim of this study was to produce a radiogallium-labeled somatostatin analog for PET studies, to characterize it and to perform initial in vivo testing.

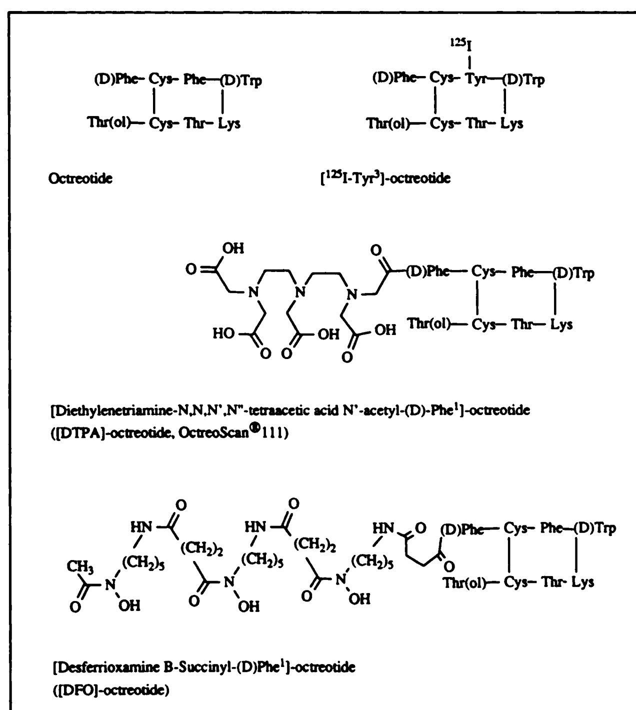

FIGURE 1. Octreotide and octreotide analogues used in this study.

MATERIALS AND METHODS

Reagents

All chemicals were obtained from Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany) unless stated otherwise. Percoll was obtained from Pharmacia (Uppsala, Sweden). Desferrioxamine B was obtained as the mesylate salt from Ciba-Geigy (Basel, Switzerland). HBED was obtained from Sigma (St. Louis, MO). Gallium(III)-67-chloride was purchased from Mallinckrodt Diagnostics (Petten, Holland). Octreotide, [DTPA]-octreotide and [^{125}I-Tyr^3]-octreotide were synthesized at Sandoz (Basel, Switzerland) according to methods given in the literature (*6,22,23*). The structures of the four peptides used are shown in Figure 1.

Instrumentation

HPLC was performed with a Hewlett Packard 1050 system with a variable wavelength UV monitor and a Raytest RSM 100 radioactivity monitoring analyzer coupled to a Compaq 386 computer operating Chromasoft software.

Activity measurements were performed with three different apparatuses depending on the radionuclidic purity, activity and physical form of the source. An 84-cm^3 high purity germanium detector (n type) in conjunction with a 916 MCB card (EG & G ORTEC, Oak Ridge, TN) installed in an IBM PC-AT was used to collect the gamma-ray spectra. Analysis of the spectra was performed using GELIGAM software (EG & G ORTEC, Oak Ridge, TN). Tracer amounts of radioisotopically pure radionuclides were assayed using a Packard A5000D automatic NaI(Tl) detector previously calibrated with sources assayed with a HPGe. Large amounts of radioisotopically pure radionuclides were determined using a Picker Microcal ionization chamber using a preset calibration factor.

Fast atom bombardment mass spectroscopy (FAB) was performed with a VG 70-SE spectrophotometer and ^{1}H nuclear magnetic resonance was performed with either a Bruker spectrophotometer at 360 MHz or a Varian VXR 400 at 400 MHz.

Synthesis

[DFO]-octreotide was synthesized in a four-step synthesis using octreotide (SDZ 201-995) and desferrioxamine B mesylate as starting materials. The overall reaction scheme is shown in Figure 2.

Octreotide was reacted with an equimolar ratio of di-t-butyldicarbonate ($(Boc)_2O$) in DMF for 2 hr at ambient temperature. The [ε-t-butyl-carbonyl-Lys^5]-octreotide (Boc-octreotide) product was separated from the di-Boc-octreotide and unreacted octreotide by silica gel column chromatography. Boc-octreotide was further reacted with an equimolar ratio of succinic anhydride in

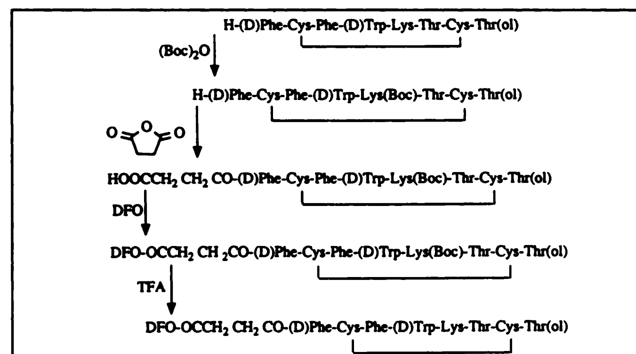

FIGURE 2. Reaction scheme for the synthesis of [Desferrioxamine B-succinyl-(D)Phe^1]-octreotide.

the presence of an excess of n-ethyldiisopropylamine to give the [succinyl-DPhe[1], Boc-Lys[5]]-octreotide which was purified by silica gel chromatography. Desferrioxamine B mesylate was converted to freebase form by ion exchange using the weak anionic exchanger AG4-X4 (Bio Rad, Richmond, CA). Conjugation of desferrioxamine B to the exposed succinate group, under "peptide bond formation" conditions (DMF as solvent and DCCI/HOBT as activators) gave Lys[5] protected DFO-octreotide. After deprotection with trifluoroacetic acid (TFA) and subsequent purification on silica gel and desalting on Duolite® S861, pure and homogeneous [DFO]-octreotide was obtained. The structure of the compound was confirmed by ^{1}H-NMR, FAB-MS and amino acid analysis.

The bifunctional HBED derivative, 1-(4-nitrobenzyl-N,N'-bis(2-hydroxylbenzyl)-ethylenediamine-N,N'-diacetic acid (NBHBED), was synthesized as outlined in the literature (*24*) and gave satisfactory analytical results.

The cold Ga[DFO]-octreotide complex was synthesized by mixing 33.3 μl 250 μM [DFO]-octreotide (0.1 *M* NaOAc pH 5.5), and 10 μl of 1.2 m*M* $Ga(NO_3)_3$ (0.1% AcOH) and the solution left at 37°C for 1 hr. The solution obtained was diluted to 1 ml with 10 m*M* HEPES buffer (pH 7.6) and filtered with a 0.22-μm filter after 24 hr. A similar procedure was used to synthesize the cold Fe[DFO]-octreotide complex and the formation of a 1:1 Fe[DFO] complex was confirmed by measuring the UV absorption at 426 nm ($\varepsilon = 2400\ M^{-1}$ cm) (*25*).

Kinetics of Ga^{3+} Incorporation into [DFO]-Octreotide

Portions (1, 2 and 5 μl) of a 500-μM [DFO]-octreotide solution (0.1 *M* NH_4OAc, pH 5.0) were added to 200 μl of a Ga^{3+} stock solution (500 n*M* Ga^{3+}, 0.1 *M* NH_4OAc, pH 5.0 spiked with $^{67}GaCl_3$). After various time intervals, portions were removed and analyzed by HPLC (C18 column, 20 m*M* NH_4OAc, pH 4.5, gradient: 0–10 min, 0% MeCN, 10–15 min, 0%–45% MeCN, 15–20 min, 45% MeCN). One milliliter eluant fractions were collected and assayed by an automatic NaI(Tl) counter. The total amount of activity recovered was >99% of that injected into the HPLC system.

[DFO]-Octreotide Labeling with Gallium

Gallium-67. A 2.3-μl portion of $^{67}Ga^{3+}$ (15 GBq/ml, 0.8 *M* HCl) was added to 100 μl of 0.1 *M* NH_4OAc (pH 6.0) together with 2 μl of a 0.5 m*M* [DFO]-octreotide (0.1% AcOH). After 20 min a 1-μl portion was removed and analyzed by HPLC (C18 column and 20 m*M* NH_4OAc, 0%–45% MeCN gradient system). After 20–60 min the labeling was absolute (>99.9%) and specific activities were generally >33 GBq ^{67}Ga/μmole [DFO]-octreotide.

Gallium-68. A commercial Al_2O_3 based ^{68}Ge/^{68}Ga (148 MBq) generator (New England Nuclear, N. Billerica, MA) was eluted with 10 ml of 5 m*M* EDTA (pH 8) at a flow rate of 1 ml/min. The eluate was added to 15 ml of 12 *M* HCl and Ga^{3+} extracted into 20 ml diethyl ether as the $H^+(GaCl_4)^-$ complex. The aqueous layer was rejected and the ether layer washed with 3 × 10 ml of 6 *M* HCl to remove traces of EDTA. After the final washing, the ether layer was evaporated to dryness and the residue was taken up in 300 μl 0.1 *M* NH_4OAc (pH 4.5). Typical generator eluted ^{68}Ga(EDTA)- and purified $^{68}Ga(OAc)_3$ yields were 16 MBq and 13 MBq, respectively, at the time of measurement.

The ^{68}Ga[DFO]-octreotide complex was formed by adding 1.5 μl of 0.5 m*M* [DFO]-octreotide (0.1% AcOH) to the $^{68}Ga(OAc)_3$ solution and waiting 5 min before application. Quality control was performed with HPLC, as above, or by TLC using silica gel impregnated glass fiber and a pyridine-to-ethanol-to-water ratio of 1:2:4 (Rf, $Ga[EDTA]^-$:0.64; $GaCl_3$:0.06; $Ga(OAc)_3$:0.08; Ga[DFO]-octreotide :0.21). Specific activities were typically 15 GBq ^{68}Ga/μmole [DFO]-octreotide.

Gallium[DFO]-Octreotide Stability Studies

Radiopharmaceutical Stability. One-hundred and five microliters of a formulation of ^{67}Ga[DFO]-octreotide (9.7 μM [DFO]-octreotide, 30 MBq $^{67}Ga^{3+}$, 0.1 *M* NH_4OAc, pH 5.5) was stored at 4°C for 20 hr. Samples were periodically removed and analyzed by HPLC (C18 column and 20 m*M* NH_4OAc, 0%–45% MeCN gradient system). One-milliliter fractions of the eluate were taken and counted after collection and after a decay period of 3 wk.

Serum Stability Studies

DFO, [DFO]-octreotide, HBED, NBHBED, DTPA and [DTPA]-octreotide were radiolabeled with $^{67}Ga^{3+}$ by incubating 2.5 μl of $^{67}Ga^{3+}$ stock solution (11 GBq/ml, 0.8 *M* HCl) with 1 μl of the ligand solution (6 m*M*) in 100 μl of acetate buffer (100 m*M*, pH 6.0) for 2 hr. Either 10 or 50 μl of the radiolabeled complex (pH 5.5) was then added to 2 ml of sterile human serum previously equilibrated in a 5% CO_2 (95% air) environment at 37°C. This solution was stored in this environment at 37°C and 150-μl aliquots were removed and analyzed after incubation periods up to 6 days. The separation of $^{67}Ga^{3+}$ bound to the ligand systems and that bound to serum proteins was performed by gel filtration on a 1 × 19-cm column of sephadex G150 or G50 resin using an elution medium of 0.1 *M* phosphate buffer (pH 7.0) at a flow of 1 ml/min and collecting 1-ml fractions which were subsequently analyzed with an automatic NaI(Tl) gamma counter. The amount of activity recovered from the column was >95% of that applied.

Whole Blood Stability and Distribution

The nonspecific binding of ^{67}Ga[DFO]-octreotide to erythrocytes and leukocytes was examined by isolating these cells as described previously (*26,27*). Briefly, 40 ml of fresh whole blood obtained from a healthy volunteer was treated with dextran before 50 μl of ^{67}Ga[DFO]-octreotide (0.24 n*M* [DFO]-octreotide) was added. This mixture was incubated at 37°C in a 5% CO_2 (95% air) environment for 1 hr with periodic mixing. The blood was then allowed to settle for 1.5 hr and plasma-containing leukocytes separated on a Percoll (polyvinylpyrrolidone-coated colloidal silica) 61%/81% density gradient column by centrifugation. The erythrocytes were washed four times with isotonic (0.9%) saline. Both the erythrocytes and leukocytes were counted using an automatic NaI(Tl) counter.

Receptor Binding Studies

The binding affinities of [DFO]-octreotide (either unlabeled or labeled with ^{nat}Ga or ^{nat}Fe) and octreotide were determined by a competition binding assay using rat cortex membranes and [^{125}I-Tyr[3]]-octreotide as a specific ligand (*28*). Typically, 20000 cpm of [^{125}I-Tyr[3]]-octreotide (6 fmoles), 50 μg of cortex membranes and the octreotide derivative (1 μM to p*M*) were incubated at room temperature for 30 min. The membranes were then isolated by rapid filtration through glass fiber filters and the activity assayed in an automatic well counter. For each data point, triplicates were performed, averaged and the data analyzed by a competition-curve analysis. Nonspecific binding was defined as the amount of activity binding to the membranes in the presence of 1 μM cold peptide.

In Vivo Evaluation

Gallium-67[DFO]-Octreotide Biodistribution and Imaging Studies. Male Lewis rats (250 ± 30 g) bearing an endocrine pan-

creatic islet cell tumor (*29*) were kindly provided by Prof. C. Wollheim (Geneva, Switzerland). These animals, as well as normal rats, were injected with 20 MBq of ^{67}Ga[DFO]-octreotide (33 GBq/μmole), in the vena jugularis. Prior to application of the ^{67}Ga[DFO]-octreotide, the animals were anesthetized with Hexobarbital (165 mg/kg) and positioned on the head of a Picker gamma camera. The gamma camera was interfaced to a VAX computer, a low-energy collimator was used and the 20% counting window centered at 180 keV. The animals were subsequently imaged at times of 1, 10, 30 and 60 min postinjection.

After a period of 1 hr, the animals were killed by cervical dislocation and the organs of interest excised, blotted and counted for ^{67}Ga activity. The injected dose was determined by measuring the injection syringe before and after application, and the activity found in the organs after 1 hr expressed as the percentage of the injected dose per gram of tissue. Samples of plasma were also isolated from a clotted portion of the blood samples and separated by a G50 (Sephadex) column (see serum stability studies).

Gallium-68[DFO]-Octreotide Biodistribution PET Studies. A total of three rats, bearing somatostatin receptor-positive endocrine pancreatic tumors, were individually subjected to a ^{68}Ga[DFO]-octreotide PET scan using the ECAT 933/16-4 camera system. This system collects data in seven transaxial 8-mm adjacent planes simultaneously. The anesthetized rat was positioned sagitally in the center of the PET detector ring and injected, with 5–9 MBq of ^{68}Ga[DFO]-octreotide (15 GBq/μmole), in the vena jugularis.

Simultaneously with the injection of the ^{68}Ga[DFO]-octreotide, a series of 27 PET scans were initiated and consisted of 5 × 10-, 5 × 20-, 5 × 40-, 5 × 80-, 5 × 200- and 2 × 600-sec periods. The rat was then moved 4 mm (half the thickness of one of the seven planes) and a further scan was performed for 600 sec. The injected dose was determined by counting the syringe before and after injection. After both scans were performed, the rat was removed and immediately killed by cervical dislocation. The various organs were isolated, weighed and counted in an automatic NaI(Tl) detector and subjected to a decay correction.

Regions of interest (ROI) were placed over the heart, liver, kidney, tumor, left shoulder and bladder. The number of counts recorded was divided by the number of pixels in the specific ROI and the acquisition time. This count rate was then converted to the activity/cm^3 by dividing by: (1) the efficiency of the PET camera for ^{68}Ga, (2) a partial volume effect factor, (3) a decay correction and (4) the volume of one pixel (*30*). The uptake of activity in the various organs was calculated as the percent of injected dose per cm^3 organ.

The correction for the partial volume effect was ascertained by drawing profiles through the peak of the activity distribution in the transaxial direction of the images reconstructed with the ramp filter. The FWHM values of these profiles are related to the size of the organ/tumor. The calibration curve was determined with various spherical sources of ^{68}Ge/^{68}Ga which were scanned in a water phantom. The correction factors varied between 1.25 and 3.5.

Since the counting efficiency is not homogeneous in the axial direction and the variation is dependent on the size of the source, response functions were determined by calibration measurements with standards of various sizes. The last 600-sec scan, which was taken with the subject position moved by half the slice thickness, was used to determine the position of the organs or tumor, respectively, relative to the center of the planes. The average uptake of the two positions was used to calculate a correction factor (from 1.05 to 1.25, depending on the size of the tumor).

TABLE 1
Percentage of Gallium[DFO]-Octreotide Formation in 0.1 *M* NH_4OAc (pH 4.5)

Gallium	[DFO]-octreotide	Reaction time (min)	%Species observed: Ga(OAc)$_3$ (3.66)†	Ga[DFO]-octreotide (14.77)†	Ga$_2$[DFO]*-octreotide (12.61)†
0.1 n*M*	0.5 n*M*	20	31.6	64.7	3.1
0.1 n*M*	0.5 n*M*	150	8.5	82.7	8.2
0.1 n*M*	1.0 n*M*	20	10.2	87.0	2.5
0.1 n*M*	1.0 n*M*	150	1.2	95.7	3.2
0.1 n*M*	2.5 n*M*	20	4.5	93.9	1.5
0.1 n*M*	2.5 n*M*	150	0.5	97.7	1.6

*Proposed species.
†Retention times in minutes.

Samples of urine were collected from killed animals and the chemical form of the ^{68}Ga activity determined by HPLC using C18 column and a 20 m*M* NH_4OAc (pH 5.5)/MeCN gradient. Fractions of the eluant (0.25 ml) were collected, counted in an automatic NaI(Tl) counter and subjected to a decay correction.

RESULTS

Kinetics of Ga^{3+} Incorporation into [DFO]-Octreotide

The rate of incorporation performed in 0.1 *M* NH_4OAc (pH 4.5) showed a dependence on [DFO]-octreotide concentrations and at low [DFO]-octreotide-to-Ga^{3+} ratios, a new species—probably a doubly labeled [DFO]-octreotide—was observed (Table 1). This new species was more prevalent at the lowest [DFO]-octreotide-to-Ga^{3+} ratio (5:1) and could be analogous to the reported $Fe_2(HDFO)^{4+}$ species (*25*) which is known to exist in acidic solutions but not at a neutral pH. When the pH was raised from 4.5 to 5.5 the quantity of this second lipophilic species was reduced below detectable levels.

[DFO]-Octreotide Labeling with Gallium

Gallium-67. The reaction ratio of 36:1 ([DFO]-octreotide-to-no carrier added $^{67}Ga^{3+}$) resulted in a fast incorporation of $^{67}Ga^{3+}$ into [DFO]-octreotide (9.6 μM) at a pH of 5.5. The kit labeling procedure with $^{67}GaCl_3$ gave consistent results with incorporation rates of 99.5 and >99.9 after incubation periods of 20 and 60 min.

Gallium-68. The elution of the generator (Al_2O_3) was typically only 20%–30% efficient and the subsequent chemical work up to remove EDTA (5 m*M*) produced an activity of around 16 MBq for labeling purposes.

The labeling of [DFO]-octreotide with $^{68}Ga^{3+}$ was optimal after 5 min and was only ca 98% complete. In general, longer incubation periods produced more hydrophilic species (eluting with the solvent front) which were observed in the HPLC activity profile. After incubation periods of 40 min, only about 96.5% of the activity was incorporated into intact [DFO]-octreotide. This decreased to 95% after 70 min and was probably due to radiolysis effects. No $^{68}Ga(DFO)^+$ was observed in HPLC elution profiles pro-

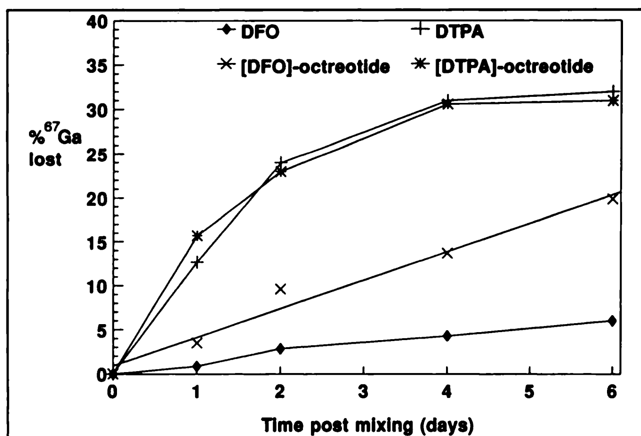

FIGURE 3. Serum stability of ^{67}Ga-labeled chelators (30 n*M*) in human serum over a 6-day period at 37°C in an environment containing 5% CO_2.

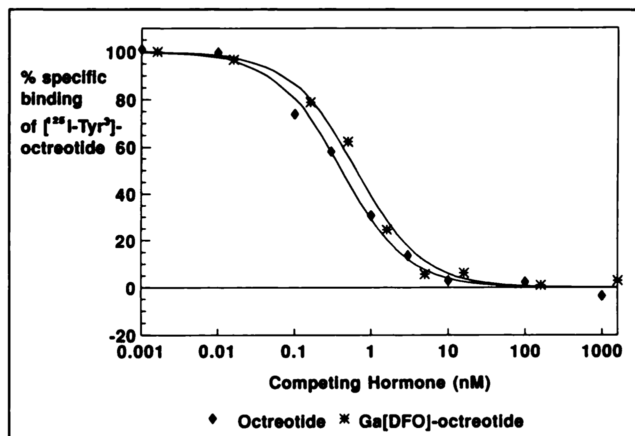

FIGURE 4. Representative binding curves for ^{67}Ga[DFO]-octreotide and [^{125}I-Tyr^3]-octreotide against octreotide with SRIF receptors expressed by rat cortex membranes.

duced 70 min postpreparation of ^{68}Ga/[DFO]-octreotide indicating the stability of the succinate linker used to couple DFO to octreotide.

Gallium-67[DFO]-Octreotide Stability Studies

Radiopharmaceutical Stability. No change in the HPLC activity profile was noted over a 24-hr period for the ^{67}Ga-labeled complex. Free gallium was consistently below 0.01% and no radiolysis assisted breakdown products were observed.

Serum Stability Studies

The stability of the monofunctional ligand complexes over a 6-day period demonstrated the high stability of both $^{67}Ga(DFO)^+$ and $^{67}Ga(HBED)^-$ with the sixth day loss of $^{67}Ga^{3+}$ to serum proteins being only 6.0% and 3.7%, respectively. Gallium-67$(DTPA)^{2-}$, by comparison, lost $^{67}Ga^{3+}$ to serum proteins to the extent of 32% within 6 days (Fig. 3). More important is the respective 24-hr values in which 0.8%, 2.7% and 12.7% of the $^{67}Ga^{3+}$ was lost to serum proteins. The Ga^{3+} complex with the bifunctional HBED demonstrated a loss of 1.3% by 24 hr and 4.6% by the sixth day. The 24-hr values for ^{67}Ga loss from ^{67}Ga[DFO]-octreotide and ^{67}Ga[DTPA]-octreotide were 3.5% and 15.7%, respectively.

The final equilibrium between the radiolabeled ligand and serum proteins was reached quicker with HBED, bifunctional HBED, DTPA and [DTPA]-octreotide as compared to DFO and [DFO]-octreotide. This would seem to indicate a higher kinetic stability of $^{67}Ga(DFO)^+$ complex over the $^{67}Ga(HBED)^-$ and $^{67}Ga(DTPA)^{2-}$ complexes. These observations are consistent with the observed efficiency of these chelators when used to treat iron overload in humans (*20,21*).

Whole Blood Stability and Distribution

The leukocytes isolated from whole blood after a 2.5-hr incubation with ^{67}Ga[DFO]-octreotide contained <0.0085% of the initial ^{67}Ga activity added while the red cells contained <0.015%. Increasing the concentration of ^{67}Ga[DFO]-octreotide had no effect on the distribution of activity indicating that the binding was nonspecific (data not shown).

Receptor Binding Studies

The competition-binding curves of octreotide, [DFO]-octreotide, ^{nat}Fe[DFO]-octreotide and ^{nat}Ga[DFO]-octreotide against [^{125}I-Tyr^3]-octreotide gave pIC_{50} values of 9.33, 8.94, 8.91 and 9.16 (n = 3). This shows that the [DFO]-octreotide conjugate still has a high affinity for somatostatin receptors and that the affinity is only slightly reduced by the addition of a bulky side chain to the peptide. The slope of the Hill plot, which represents the number of different types of interaction, was close to unity (1 ± 0.05) indicating that there was only one type of hormone/receptor binding interaction. Figure 4 shows representative binding curves for [^{125}I-Tyr^3]-octreotide against octreotide and Ga[DFO]-octreotide.

In Vivo Evaluation

Gallium-67[DFO]-Octreotide Biodistribution and Imaging Studies. The first image obtained at 1 min postinjection already clearly showed the tumor together with the heart,

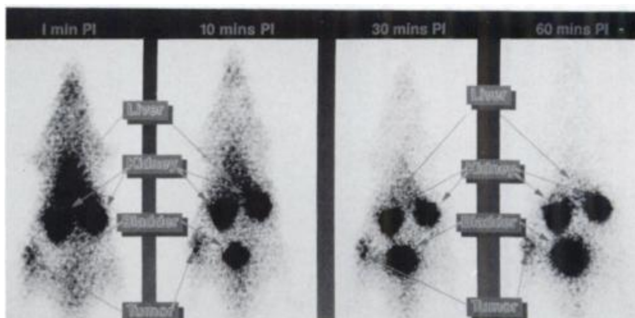

FIGURE 5. Planar gamma camera images of a rat bearing an islet cell tumor 1, 10, 30 and 60 min postinjection with 20 MBq of ^{67}Ga[DFO]-octreotide.

TABLE 2
Biodistribution of ^{67}Ga[DFO]-Octreotide One Hour Postinjection in Normal and Islet Cell Tumor-Bearing Rats Injected with 20 MBq ^{67}Ga[DFO]-Octreotide (33 GBq/μmole)

Tissue	Control rats %ID/g ± s.e.m.* (n = 5)	Tumor-bearing rats %ID/g ± s.e.m.* (n = 4)
Blood	0.25 ± 0.05	0.16 ± 0.04
Muscle	0.07 ± 0.02	0.07 ± 0.05
Heart	0.08 ± 0.04	0.05 ± 0.01
Kidney	4.99 ± 0.95	3.31 ± 0.58
Liver	0.26 ± 0.02	0.21 ± 0.04
Spleen	0.18 ± 0.04	0.14 ± 0.03
Intestine	0.21 ± 0.05	0.27 ± 0.09
Tumor		0.38 ± 0.08
Ratios		
Tumor-to-blood		2.5 ± 1.1
Tumor-to-muscle		7.4 ± 4.7
Tumor-to-liver		1.9 ± 0.8
Tumor-to-intestine		1.6 ± 0.6

*s.e.m.: standard error of the mean.

liver and kidneys (Fig. 5). Subsequent images obtained up to 1 hr postinjection show a progressive clearance of activity from the blood pool and after 1 hr only the tumor, kidneys and bladder are clearly visible. The biodistribution data for both normal and endocrine tumor-bearing rats (Table 2) showed low binding to normal tissue and a tumor-to-muscle ratio of 5 was found after 1 hr. The major excretion route was the kidneys but the accumulation of activity in the liver and intestine suggests that a small percentage of the compound is cleared by these organs. The analysis of the plasma samples showed that only 0.2% of the injected dose was present and bound to a high molecular weight serum protein (i.e., transferrin) 1 hr postinjection.

Gallium-68[DFO]-Octreotide Biodistribution PET Studies. The images obtained 1 hr postinjection with 5–9 MBq of ^{68}Ga[DFO]-octreotide (15 GBq/μmole) clearly show the activity located in the tumor, kidney and bladder (Fig. 6). The liver is also visible, partly because of ^{68}Ga remaining in the blood pool. These images were used to construct regions of interest (ROI) in each plane and, by measuring the number of counts in each ROI for all the scans, the dynamic biodistribution of ^{68}Ga[DFO]-octreotide could be determined over the 2-hr scan.

The tumor was visible (with PET) within 30 sec postinjection and a slight increase in localized activity was noted for up to 1000 sec (maximum 0.9% ID/ml) thereafter a k_{off} rate of $3.0 \pm 0.5 * 10^{-5}\ s^{-1}$ was observed (Fig. 7). The activity in the kidney peaked 200–300 sec postinjection (3.3% ID/ml) and was accompanied by a shift of activity to the bladder. The biodistribution data obtained from the sacrificed rat directly after the last PET scan showed a good correlation with the PET data (Table 3).

Analysis of the urine excreted by rats injected with ^{68}Ga[DFO]-octreotide showed that the labeled peptide was excreted intact (data not shown).

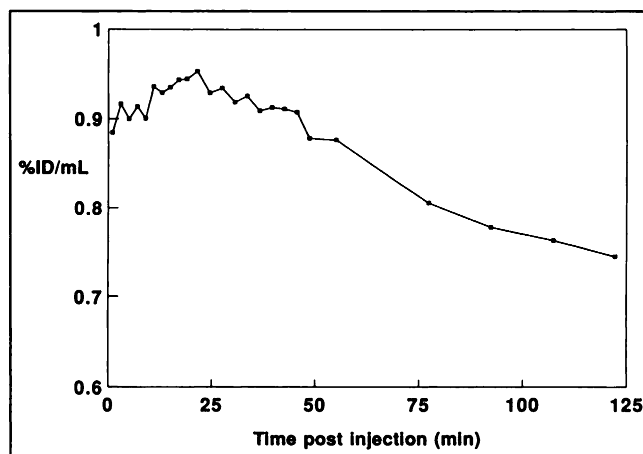

FIGURE 7. PET-computed tumor accumulation and release of ^{68}Ga[DFO]-octreotide in a rat bearing an islet cell tumor.

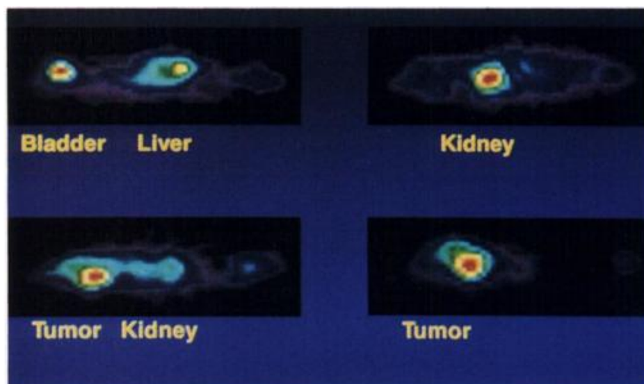

FIGURE 6. A series of PET-computed sagittal planes from a rat bearing an islet cell tumor on its left flank taken 1 hr postinjection with 8 MBq of ^{68}Ga[DFO]-octreotide.

TABLE 3
Comparison of Biodistribution Data and PET Data in Islet Cell Tumor-Bearing Rats Injected with ^{68}Ga[DFO]-Octreotide (n = 1)

Dose (μg)	Tissue	Time of last scan*	%ID/g Biodistribution	%ID/ml PET data
1.1	Tumor	1.91	0.76	0.74
	Muscle		0.11	0.04
	Kidney		2.18	1.80
2.2	Tumor	2.50	0.28	0.27
	Muscle		0.07	0.11
	Kidney		2.33	1.73
1.2	Tumor	1.00	0.56	0.44
	Muscle		0.11	0.04
	Kidney		1.02	1.16

*Hours postinjection.

DISCUSSION

The introduction of [^{123}I-Tyr^3]-octreotide (*7*), in particular Octreoscan®111 (*8*), has led to the effective imaging of SRIF receptor-positive tumors in man. The introduction of a gallium-labeled somatostatin analog is intended primarily as a PET agent for the imaging of SRIF receptor-positive tumors. Gallium-68 is a generator radionuclide whose 68-min half-life and 89% decay by β^+ emission make it well suited for PET studies. Additionally, the use of DFO as a chelating agent enables the chelation and subsequent PET studies with ^{52}Fe ($T_{1/2}$ 8.27 hr). This would enable the long-term (24 hr) study of the pharmacokinetics of somatostatin receptors to be performed (i.e., tumor K_{off}).

The ^{67}Ga and ^{68}Ga labeling was optimal at pHs of 5.5 and 4.5, respectively. The labeling of DFO with Ga^{3+} is pH dependent. The solubility of $Ga(OH)_3$ is also pH dependent. The two pH values used reflect the weak complexing ability of acetate buffer and the concentrations of Ga(III) (i.e., 10 mCi ^{67}Ga/ml = 250 n*M* Ga(III), 10 mCi ^{68}Ga/ml = 2.2 n*M* Ga(III)). A higher specific activity for both radiopharmaceuticals is possible but only feasible at a higher pH where the formation of stable $Ga_2(HDFO)^{4+}$ dimers is thermodynamically unfavorable (*25*). The ^{68}Ga labeling procedure could be much improved by the use of a SnO_2-based ^{68}Ge/^{68}Ga generator system (*31*) which would allow the elution of $^{68}GaCl_3$ and avoid the wet chemistry required to convert ^{68}Ga[EDTA]$^-$ to $^{68}Ga(OAc)_3$. This improvement would also reduce the inevitable Fe contamination (from reagents, glassware, etc.) and improve the final specific activity of the radiopharmaceutical.

The kit type procedure developed for $^{67}Ga^{3+}$ labeling of [DFO]-octreotide is fast in achieving a very high radiochemical purity and consequentially it requires no purification prior to injection. In terms of specific activity, both the ^{67}Ga[DFO]-octreotide (33 GBq/μmole) and ^{68}Ga[DFO]-octreotide (15 GBq/μmole) are comparable to the ^{111}In-[DTPA]-octreotide (17–25 GBq/μmole) (*32*) and [^{123}I-Tyr^3]-octreotide (11–19 GBq/μmole) (*7*).

The ^{67}Ga[DFO]-octreotide, once prepared, is stable for periods up to 24 hr at 4°C and, unlike ^{111}In[DTPA]-octreotide, it was not susceptible to radiolysis (*23*). This unexpected result for the two radiopharmaceuticals of similar specific activities and whose radioisotopes have similar decay energies could be explained by the better protection afforded by DFO to the peptide. In contrast, the ^{68}Ga[DFO]-octreotide suffered considerable radiation damage when left in the labeling buffer for prolonged periods of time. The main breakdown product elutes from the C18 column at the solvent front and consequently it is unlikely to be ^{68}Ga still conjugated to DFO, but rather conjugated to a fragment of the DFO.

The serum stability test used was a static evaluation of what should happen in serum, but in practice, the process is dynamic with a true equilibrium never really being obtained so that the kinetic data are more important than the final equilibrium result. The observed kinetic stability order was DFO > [DFO]-octreotide > DTPA > [DTPA]-octreotide > HBED > NBHBED. The reports in the literature support this concept, with the ligand DTPA showing a rapid loss of Ga^{3+} (*16*) and the highly thermodynamically stable ligand HBED also showing a higher loss (*19*) than would be expected from the conditional stability constants alone (*33,34*).

The serum stability tests indicated that DTPA could have some use as a chelating agent for $^{68}Ga^{3+}$ labeling of peptides, but a recent report (*35*) shows a relatively slow incorporation rate of $^{68}Ga^{3+}$ into DTPA coupled to a chemotactic peptide (ca 40% within 30 min). HBED was not investigated further as a ligand for ^{67}Ga because of the insoluble nature of the HBED-octreotide conjugate which could not be isolated and purified.

The initial biodistribution studies showed a tumor accumulation of 0.38% ID/g after 1 hr. The tumor-to-nontumor ratios achieved after 1 hr (blood, liver, intestine and muscle ratios of 2.5, 1.9, 1.6 and 7.4, respectively) were similar to those obtained with ^{111}In[DTPA]-octreotide (comparable ratios 1.7, 1.8, 4.2 and 5.0, respectively) (*32*). The main excretion route was via the kidneys, and in that respect quite similar to ^{111}In[DTPA]-octreotide (*32*). The blood clearance appeared to be faster than that of ^{111}In[DTPA]-octreotide (*32*) but this is offset by the higher intestinal uptake observed after 1 hr.

The initial PET studies did, however, demonstrate the first selective targeting of a receptor using a hormone labeled with a metallic positron emitter, and thus it enabled the %ID/ml at various organs to be determined over a period of time. In the three animals studied, the observed residence half-life of ^{68}Ga[DFO]-octreotide at the tumor was about 6 hr. The receptor density of this tumor line is ca 100–200 fmoles/mg and so the tumor accumulation of >1% of the injected dose (600 pmoles) does not represent a total saturation of receptors at the tumor. Further studies (data not shown) have indicated that there is no significant variation in the biodistribution (%ID/g) of radiolabeled octreotide even when the injected dose is raised to 6 nmoles/animal.

Further studies have been performed to further characterize the in vitro and in vivo properties of ^{67}Ga[DFO]-octreotide with another tumor model and will be presented (*36*). Similarly, further PET studies are in progress with this tumor line with both ^{68}Ga and ^{52}Fe. Iron-52, like ^{68}Ga, forms kinetically stable complexes with DFO. The major benefits of this new radiopharmaceutical are that it enables the selective targeting on a receptor with a positron-emitting metallic radionuclide.

Radiolabeled somatostatin analogs have considerable benefits over both monoclonal antibodies and metabolic markers (^{18}F-2-fluoro-2-deoxy-D-glucose, ^{11}C-methionine) for the detection and staging of somatostatin receptor-positive tumors. Monoclonal antibodies are notoriously slow both to clear from the blood pool and to accumulate at the tumor. More importantly is the induced immune response (human anti mouse antibody, HAMA) initially reported

with murine antibodies (*37,38*) and more recently reported with chimeric antibodies (*39*) which were specifically designed to avoid a HAMA response. PET metabolic markers as the name implies are not specific.

The chemistry involved is simple and when used in conjunction with a $^{68}Ge/^{68}Ga$ generator eluting $^{68}GaCl_3$, it enables a reliable lyophilized kit to be used to label the [DFO]-octreotide. In comparison, organic PET radiopharmaceuticals (i.e., ^{18}F-octreotide (*40*) or ^{11}C-octreotide) would require multiple steps and a purification step to remove various byproducts. The former requires little skill or apparatus whereas the latter requires a skilled radiochemist or a sophisticated level of automation in addition to a specialized airtight working environment.

In conclusion, ^{68}Ga[DFO]-octreotide is a radiometal-labeled hormone analogue which can be used for PET studies with receptor-positive tumors. In the clinic, its simple kit formulation, from a generator nuclide, would greatly simplify the PET diagnosis and staging of such SRIF receptor-positive tumors as gastroenteropancreatic, small-cell lung and breast tumors.

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