High Specific Activity [Samarium-153] EDTA for Imaging of Experimental Tumor Models

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Enriched samarium oxide (98.2% $^{152}Sm_2O_3$) was irradiated in low neutron flux and high neutron flux reactors to produce ^{153}Sm with specific activities of 14.3 GBq and 22.1 TBq $mmol^{-1}$ Sm, respectively, at the time of use. Formulation of ^{153}Sm as [^{153}Sm]EDTA, with a 1:10 molar ratio of SM:EDTA, provided a stable radiotracer in vitro and in vivo. High specific activity [^{153}Sm]EDTA showed superior uptake in cell culture (20.8 ± 0.9% vs. 5.5 ± 0.1% for 6 and 600 pmol Sm per 10^6 cells, respectively) and better tumor index values (51 vs. 37 at 10.9 nmol and 1.09 μmol Sm kg^{-1}, respectively) in the BDF_1 mouse/Lewis lung tumor model. High specific activity [^{153}Sm]EDTA scintigrams of Copenhagen × Fisher rats bearing transplanted Dunning R3327 tumors clearly delineated the tumors within 6 hr, with moderate liver and bone uptake and low soft-tissue background. The injected radiotracer underwent rapid central compartment clearance and whole-body elimination. The absence of observed adverse histopathological toxicity combines with high image quality within 6 hr, to support the clinical tumor-imaging potential of this agent. Comparative studies with [^{67}Ga]citrate at molar-equivalent doses indicated that high specific activity [^{153}Sm]EDTA was a superior radiotracer in these in vitro and in vivo models.

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The known tumor affinity of Group III elements such as gallium and indium has stimulated interest in the use of radiolanthanides and radioactinides for both radiotherapy and scintigraphy. Gallium-67 (^{67}Ga) and indium-111 (^{111}In) have found broad application in diagnostic nuclear medicine (*1,2*). The use of radiolanthanides, including samarium-153 (^{153}Sm) for tumor imaging was pioneered two decades ago (*3*). Although O'Mara et al. (*3*) proposed that the radiolanthanides would be useful bone scanning agents, ^{153}Sm has recently received attention as a therapeutic radiopharmaceutical for the palliative treatment of metastatic bone cancer (*4–6*). In spite of the complex decay scheme which includes various beta (E_{max} 0.8 MeV) and electron (to 0.1 MeV) radiations (*7*) associated with ^{153}Sm decay, this reactor-produced radiolanthanide emits gamma radiations which are well-suited for high efficiency, high resolution gamma cameras. Favorable tumor uptake has been demonstrated for several radiolanthanides and a number of ^{153}Sm tumor uptake studies have been reported (*8–15*).

Samarium-153 is a reactor-produced radiolanthanide that can be obtained in high yield and with high specific activity from low-cost, enriched $^{152}Sm_2O_3$. Radioactive decay yields a principal gamma photon of 103 keV (29.8%), with little (0.6%) high-energy gamma emission and a half-life of 46.27 hr (*7,16*). Quantitative tumor uptake and scintigraphy of high specific activity [^{153}Sm] ethylenetriaminetetraacetic acid (EDTA) in experimental tumor models are now reported, in comparison with a lower specific activity formulation and with [^{67}Ga] citrate.

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METHODS AND MATERIALS

Production of ^{153}Sm

Low specific activity [^{153}Sm]samarium oxide (1.1 mCi mg^{-1} Sm_2O_3; 14.3 GBq.$mmol^{-1}$) was produced by thermal neutron irradiation (4 hr at 1×10^{12} $n \cdot cm^{-2} \cdot sec^{-1}$) of enriched [$^{152}Sm$] Sm_2O_3 (Oak Ridge National Laboratory; 98.2% ^{152} Sm) at the University of Alberta SLOWPOKE Reactor Facility. High specific activity ^{153}Sm was produced by irradiation (7 days at 2×10^{14} $n \cdot cm^{-2} \cdot sec^{-1}$) of the enriched Sm_2O_3 at the Atomic Energy of Canada Ltd. research reactor facility (AECL Commercial Products Division, Chalk River, Canada). The specific activity at time of use was estimated to be 1.7 Ci mg^{-1} Sm_2O_3 (22.1 TBq $mmol^{-1}$ Sm). Yields and radionuclidic purity were similar to those reported by Ehrhardt et al (*17*). Radionuclidic purity was determined by high resolution Ge(Li) gamma

spectrometry. The 70- and 103-keV photopeaks of ^{153}Sm were the main gamma emissions detected, along with low-energy photons arising from trace amounts of unidentified radionuclidic contaminants. Radionuclidic purity was estimated to be > 99%, based on gamma counts at the time of use.

Preparation of [^{153}Sm]EDTA

Irradiated [^{153}Sm]Sm_2O_3 was dissolved in sufficient 1N HCl to produce a solution of 1 mg $SmCl_3$ ml^{-1}. This was added to an aqueous solution of disodium ethylenediaminetetracetic acid (Na_2EDTA) so that the molar ratio was Sm:EDTA = 1:10. After standing for 30 min, the pH was adjusted to 6.5 by slow, careful addition of 1N NaOH, after which the solution was filtered through a 0.22-μm filter (Millipore Ltd., Mississauga, Canada). These solutions were diluted with distilled water to a final activity of 370 kBq in 0.1–0.2 ml.

Samarium-153 carbon-14 (^{14}C) EDTA was prepared by mixing the [^{153}Sm]$SmCl_3$ solution (37 MBq) with a solution of [^{14}C]EDTA in 0.1N HCl (370 kBq; 4 GBq $mmol^{-1}$) (Amersham Canada Ltd., Oakville, Canada). After incubation for 30 min, an aqueous solution of Na_2EDTA was added to make a solution with a molar ratio Sm:EDTA = 1:10. After an additional 30 min the pH was adjusted to 6.5 with 1N NaOH and filtered through a 0.22-μm filter. Stability of this formulation, as judged by colloid formation in RPMI growth medium, was determined by ultracentrifugation as reported elsewhere (*14*).

Gallium-67 citrate (Merck Frosst Inc., Montreal, Canada) was obtained as a sterile, non-pyrogenic solution containing 9 ng Ga ml^{-1} with a concentration of 2 mCi (74 MBq) ml^{-1}. Carrier Ga citrate was added for in vitro studies so that uptake could be compared with Sm uptake at identical molar concentrations.

Radiochromatography and Radiometry

Radiochemical purity of the radiotracer formulation was determined using ascending paper chromatography. Strips of Whatman No. 1 paper (25 × 130 mm) were run in pyridine:ethanol:water = 1:2:4 for 2 hr, after which they were dried, cut into transverse strips (10 mm) and counted in a well-type gamma counter (Smith Kline Beckman, Irvine, CA; Model 8000). Cellulose gel thin layer chromatography (TLC) (Terochem Laboratories, Edmonton, Canada; Eastman Kodak 6064 plates) were also run with this solvent system. After development, the chromatograms were cut into 10-mm strips and counted in a gamma counter. In the case of dual-label ($^{153}Sm/^{14}C$) studies, the strips were re-counted after 21 days by adding distilled water (1.5 ml) to wet the strip, letting it stand overnight and then mixing with scintillation fluor (DuPont Company, Inc., Montreal, Canada; Aquasol II) prior to beta counting in a liquid scintillation counter (Smith Kline Beckman, Irvine, CA; Model 9000). The stability of [^{153}Sm]EDTA was determined in the presence of [^{45}Ca]calcium chloride (Terochem) (1.63 MBq μg^{-1}). The solutions were mixed, allowed to stand for 30 min and then applied to thin layer chromatographic (TLC) plates and analyzed as described above.

In vitro cell uptake studies

Human Melanoma 2AB and murine EMT-6 cell lines were obtained from the Cross Cancer Institute, Edmonton. The Melanoma 2AB cells were maintained in vitro as monolayers in RPMI-1640 medium which contained heat-inactivated fetal calf serum (FCS 15%) and glutamine (0.29 mg ml^{-1}). The EMT-6 cells were maintained in vitro as a monolayer in Waymouth's medium supplemented with FCS (10%) and glutamine (0.29 mg ml^{-1}). Suspensions of the respective cell lines for in vitro studies were prepared by washing monolayer cells with Versene buffer, followed by a brief incubation (1 min) with 0.1% trypsin (Gibco, Grand Island, NY). Cells were then washed (2 × 10 ml) with the respective growth medium, followed by dilution to 1 × 10^6 cells ml^{-1} in that medium. Cell viability was determined by the trypan blue exclusion test as described elsewhere (*15*). Viability normally exceeded 98%. In in vitro uptake studies, cell suspension aliquots (1 ml; 1 × 10^6 cells) were incubated with the radiotracer formulation (0.6 nmol radionuclide for Melanoma 2AB cells and 0.006 nmol radionuclide for EMT-6 cells) at 37° for predetermined intervals. After incubation, cells were pelleted by centrifugation and washed twice with normal saline, and then both the cell pellets and the combined supernatants were radioassayed. Experiments were run in triplicate, with corrections for nonviable cells.

In vivo biodistribution of ^{153}Sm and ^{67}Ga radiotracers

Whole-body tissue distribution studies were carried out on male BDF_1 mice (28–30 g) bearing Lewis lung carcinoma which had been implanted in the flank. Radiotracer solutions (370 kBq in 0.1–0.2 ml) were slowly injected via the tail vein at mass doses of 1.09 μmol and 10.9 nmol kg^{-1} (for low and high specific activity formulations, respectively) into mice which had tumors with diameters of 5–7.5 mm.

At specified time intervals, animals were killed; an aliquot of blood and the entire lungs, liver, spleen, kidney, femur and tumor were collected, weighed and radioassayed using a well-type gamma scintillation counter. The remaining carcass was counted in a large-volume well counter (Picker International, Northford, CT; Model Autowell II). In mice dosed with [^{153}Sm][^{14}C]EDTA, tissue specimens weighing ~ 60 mg were taken for gamma analysis, then stored for 40 days (for ^{153}Sm decay) prior to radioassay for ^{14}C. These samples were solubilized in a mixture of 2 ml Protosol (Amersham) in 100 μl water. After dissolution, samples were decolorized by the addition of a few drops of hydrogen peroxide (30% v/v), neutralized by addition of glacial acetic acid (0.1 ml) and mixed with scintillation fluor (DuPont Company) (15 ml). Stannous chloride (three drops of a 4% w/v solution in 0.1N HCl) was added to reduce chemiluminescence, and samples were dark-adapted at 5° for 30 min prior to counting in a liquid scintillation counter. Counting efficiency corrections were based on the H-number external standard (Smith Kline Beckman), with interpolation from a set of quenched standards prepared in an identical manner but containing a known amount of [^{14}C]hexadecane (Amersham).

Whole-body scintigraphy of male Copenhagen × Fisher rats (300–325 g) bearing subcutaneously-implanted Dunning R-3327H prostatic tumors was undertaken using a gamma camera (Siemens Medical Systems, Des Plaines, IL) fitted with a high resolution collimator designed for technetium-99m. Scintigrams of not less than 100,000 counts were recorded after i.v. injection (femoral vein) of either 1.09 μmol or 10.9 nmol Sm per kg body weight (3.7 MBq) for low and high specific activity Sm, respectively. In a preliminary study, a formulation with Sm:EDTA = 1:2 was also used with a Sm

dose of 1.09 μmol per kg body weight. In some studies, rats were housed individually in metabolic cages after dosing, with food and water ad libitum. Urine was collected at various intervals for further analysis. In all cases, quantitative whole-body counting was undertaken using the gamma camera, with the rat held in fixed geometry during measurements.

Limited studies on weight gain and histopathologic effect of i.v. doses of Sm-EDTA (0.1, 1.09 and 10.9 μmol kg^{-1}) were undertaken using male ALAS mice (20–23 g). Weights were recorded on Days 1, 4, 12, 18, 24, 28, 32, 36, and 48, and histopathologic examinations were performed at Days 1, 20, 48, and 64. The male offspring of pregnant female ALAS mice dosed with i.v. [Sm]EDTA (1.09 μmol kg^{-1}) were killed 48 and 64 days after birth. Organs examined included lungs, liver, kidney, and femur. Preliminary radiodosimetry estimates were made using the MIRD method (*18*).

RESULTS

Samarium-153 EDTA was formulated with 1:10 Sm:EDTA molar concentration ratio to ensure complete chelation of ^{153}Sm. TLC of the product at pH 6.5 showed a single spot at Rf 0.9, whereas at 1:1 stoicheometry, two approximately equal spots at Rf 0.1 ($^{153}SmCl_3$) and 0.9 were present. This formulation was stable when incubated for 1 hr in RPMI growth medium, with only 3.5 ± 0.6% precipitable by centrifugation at 144,000 *g* for 1 hr; incubation in distilled water resulted in 3.3 ± 2.3% sedimentation in control experiments. All sedimentation experiments were performed in triplicate at a concentration of 6 μmol [^{153}Sm] EDTA per ml per incubation tube.

The stability of Sm-EDTA in the presence of ionic calcium, which occurs in blood in appreciable concentrations, was tested in two ways. Incubation (1 hr) of [^{153}Sm]Cl_3 with an equimolar amount of Ca-EDTA (Rf 0.9) led to formulation of [^{153}Sm]EDTA in > 90% yield, whereas incubation of [^{153}Sm]EDTA with $^{45}CaCl_2$ (Rf 0.05) resulted in no measurable formation of [^{45}Ca] EDTA.

Uptake of [^{153}Sm]EDTA by Melanoma 2AB cells in culture increased as a function of incubation time over a 2-hr period. Samarium uptake was 3.6 ± 0.5 and 5.3 ± 0.3% per 10^6 cells at 1 hr and 2 hr, respectively, compared to 3.0 ± 0.2 and 3.9 ± 0.1 for [^{67}Ga]citrate. Both radiotracers were studied at radionuclide concentrations of 0.6 nmol per ml per incubation tube. Uptake studies with low specific activity [^{153}Sm][^{14}C]EDTA showed similar uptakes for ^{153}Sm, but ^{14}C uptakes were always ~ 40% of the ^{153}Sm value. Studies with high specific activity [^{153}Sm][^{14}C]EDTA showed similar ^{14}C accumulations, but [^{153}Sm] values increased much more rapidly. Data are presented in Table 1.

Quantitative whole-body distribution of ^{153}Sm, ^{14}C, and ^{67}Ga radioactivity was compared after i.v. injection of (a) low specific activity [^{153}Sm][^{14}C]EDTA, (b) high specific activity [^{153}Sm]EDTA, and (c) [^{67}Ga]citrate, in BDF_1 mice bearing Lewis lung carcinoma. Samarium-153 and ^{67}Ga were found to be concentrated in several tissues, but in all cases, [^{14}C]EDTA accumulations were more transient and of a lower magnitude than concentrations of the other two radionuclides. Samarium-153 biodistribution at both specific activities was characterized by rapid blood clearance to very low levels (< 0.01% injected dose ml^{-1}) compared with ^{67}Ga (0.8% injected dose ml^{-1}), thereby leading to greatly elevated tumor:blood and tissue:blood ratios for ^{153}Sm compared with ^{67}Ga. Specific activity-related effects included a twofold increase in % injected dose (% I.D.) g^{-1} of tissue (tumor, liver, kidneys, lung and spleen) for a 100-fold decrease in ^{153}Sm dose, with no appreciable change in bone uptake. The high specific activity ^{67}Ga showed a four- to sixfold increase in % I.D. g^{-1} soft tissue at 1 hr, but these values converged at later intervals; however, bone uptake at low specific activity demonstrated the inverse effect, with ^{67}Ga levels nearly twice as high as those found after injection of high specific activity [^{67}Ga]citrate. Tissue:blood ratio maxima invariably occurred 48 hr after injection for ^{153}Sm, whereas the maxima always occurred at 24 hr for ^{14}C and usually at 24 hr for ^{67}Ga. Selected data are presented in Table 2.

The influence of Sm:EDTA ratios at a Sm dose of 1.09 μmol kg^{-1} whole-body weight was investigated in

TABLE 1
Uptake of [^{153}Sm][^{14}C]EDTA by Melanoma 2AB cells in Suspension. Data are Means ± s.d. for 3 Determinations

Incubation time (min)	% Radioactivity in Cells					
	$^{153}Sm^1$	$^{14}C^1$	$^{153}Sm/^{14}C^1$	$^{153}Sm^2$	$^{14}C^2$	$^{153}Sm/^{14}C^2$
5	3.7 ± 0.2	1.6 ± 0.1	2.3 ± 0.2	2.8 ± 0.7	1.8 ± 0.3	1.6 ± 0.3
10	4.3 ± 0.3	1.9 ± 0.2	2.3 ± 0.2	5.5 ± 0.2	2.2 ± 0.2	2.5 ± 0.2
20	4.7 ± 0.4	2.1 ± 0.1	2.2 ± 0.2	8.2 ± 0.1	2.2 ± 0.1	3.8 ± 0.2
30	4.9 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	9.1 ± 0.3	2.4 ± 0.3	3.9 ± 0.6
45	5.0 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	10.5 ± 2.9	2.6 ± 0.1	4.0 ± 1.1
60	5.6 ± 0.4	2.7 ± 0.0	2.1 ± 0.1	14.9 ± 3.1	2.5 ± 0.6	5.9 ± 1.4
120	5.5 ± 0.1	2.8 ± 0.1	2.0 ± 0.1	20.8 ± 0.9	3.2 ± 0.1	6.6 ± 0.7

[1] Each tube contained 0.6 nmole Sm, Sm:EDTA = 1:10 (low specific activity ^{153}Sm).
[2] Each tube contained 0.006 nmole Sm, Sm:EDTA = of 1:10 (high specific activity ^{153}Sm).

TABLE 2

Quantitative Whole-Body Distribution of High Specific Activity [^{153}Sm][EDTA, Low Specific Activity [^{153}Sm][^{14}C]EDTA and [^{67}Ga] Citrate in BDF$_1$ Mice Bearing Transplanted Lewis Lung Carcinoma. Data are Means ± s.d. for 4 Animals

Tissue	Radiotracer	Time After Injection 1	6	24	48	Tissue:blood Maximum ± s.d. (time, hr)
Blood	[^{153}Sm][^{14}C]EDTA[a]	0.22 ± 0.06[b]	0.04 ± 0.01	0.02 ± 0.00	<0.01 ± 0.00	----------
	[^{153}Sm][^{14}C]EDTA[c]	0.54 ± 0.15	0.08 ± 0.04	0.05 ± 0.02	0.43 ± 0.04	----------
	[^{153}Sm]EDTA[d]	1.60 ± 0.21	0.37 ± 0.08	0.02 ± 0.01	0.01 ± 0.00	----------
	^{67}Ga citrate[e]	3.40 ± 0.29	1.89 ± 0.25	0.81 ± 0.05	0.80 ± 0.22	----------
	^{67}Ga citrate[d]	24.51 ± 12.1	12.2 ± 0.21	2.84 ± 0.77	0.96 ± 0.25	----------
Bone	[^{153}Sm][^{14}C]EDTA[a]	12.2 ± 2.5	17.1 ± 3.21	12.9 ± 2.41	5.02 ± 1.23	867 ± 142 (48)
	[^{153}Sm][^{14}C]EDTA[c]	0.56 ± 0.16	0.13 ± 0.08	0.36 ± 0.29	0.18 ± 0.06	8.6 ± 7.7 (24)
	[^{153}Sm]EDTA[d]	12.4 ± 2.2	18.6 ± 8.1	15.9 ± 4.9	6.98 ± 0.71	853 ± 87 (24)
	^{67}Ga citrate	20.1 ± 1.9	10.3 ± 2.89	12.9 ± 2.7	15.8 ± 0.65	20 ± 5 (48)
	^{67}Ga citrate[d]	10.7 ± 1.2	7.25 ± 1.65	7.43 ± 0.77	8.77 ± 2.35	9.2 ± 2.5 (48)
Tumor	[^{153}Sm][^{14}C]EDTA[a]	0.85 ± 0.21	1.23 ± 0.31	0.74 ± 0.09	0.39 ± 0.10	65 ± 17 (48)
	[^{153}Sm][^{14}C]EDTA[c]	0.62 ± 0.28	0.24 ± 0.14	0.27 ± 0.11	0.26 ± 0.17	4.3 ± 2.2 (24)
	[^{153}Sm]EDTA[d]	1.95 ± 0.19	1.70 ± 0.14	1.01 ± 0.11	0.72 ± 0.12	72 ± 16 (48)
	^{67}Ga citrate	0.88 ± 0.09	0.76 ± 0.19	1.37 ± 0.75	1.23 ± 0.42	1.7 ± 0.9 (24)
	^{67}Ga citrate[d]	7.11 ± 3.62	5.37 ± 1.78	3.04 ± 0.67	2.09 ± 0.52	2.2 ± 0.3 (48)
Liver	[^{153}Sm][^{14}C]EDTA[a]	4.88 ± 1.71	6.17 ± 2.03	5.95 ± 0.89	4.15 ± 0.42	691 ± 65 (48)
	[^{153}Sm][^{14}C]EDTA[c]	0.72 ± 0.18	0.28 ± 0.16	0.43 ± 0.26	0.22 ± 0.12	8.7 ± 5.0 (24)
	[^{153}Sm]EDTA[d]	12.8 ± 0.83	13.1 ± 1.97	12.1 ± 1.81	7.67 ± 0.84	767 ± 88 (48)
	^{67}Ga citrate	3.09 ± 0.68	4.63 ± 1.85	17.3 ± 1.39	16.0 ± 4.12	21 ± 2 (24)
	^{67}Ga citrate[d]	15.7 ± 7.82	13.9 ± 1.41	12.5 ± 2.76	11.8 ± 2.96	12 ± 2 (48)
Kidney	[^{153}Sm][^{14}C]EDTA[a]	3.19 ± 1.12	3.02 ± 1.21	1.55 ± 0.43	0.97 ± 0.29	161 ± 53 (48)
	[^{153}Sm][^{14}C]EDTA[c]	4.41 ± 3.09	1.27 ± 1.02	0.88 ± 0.55	0.67 ± 0.07	18 ± 10 (24)
	[^{153}Sm]EDTA[d]	13.89 ± 2.08	5.99 ± 1.20	1.56 ± 1.01	1.48 ± 0.29	148 ± 30 (48)
	^{67}Ga citrate	5.07 ± 0.92	2.34 ± 1.05	2.18 ± 0.48	1.61 ± 0.52	2.7 ± 0.4 (24)
	^{67}Ga citrate[d]	16.4 ± 8.22	7.08 ± 0.71	6.25 ± 4.01	5.30 ± 1.32	5.5 ± 0.3 (48)

[a] ^{153}Sm distribution after [^{153}Sm][^{14}C]EDTA injection (1.09 mol Sm kg^{-1} body weight).
[b] % injected dose per g ± s.d.
[c] ^{14}C distribution after injection of a (above).
[d] High specific activity radiaotracer (10.9 nmol Sm or Ga kg^{-1} body weight).
[e] ^{67}Ga distribution after [^{67}Ga] citrate injection (1.09 mol Ga kg^{-1} body weight).

a preliminary scintigraphic experiment with rats bearing implanted Dunning prostatic tumors. At a 1:2 ratio of Sm:EDTA, early hepatic activity was evident along with kidney and bladder visualization, as was the case for the 1:10 formulation. However, the 1:2 formulation produced a rapid and continuing intensification of activity in the liver throughout the observation period, suggesting gradual formation of colloidal ^{153}Sm and trapping of the colloid in the liver. At the 1.09 μmol kg^{-1} dose, the 1:10 formulation provided good delineation of the skeleton, liver and the tumor within 6 hr.

High specific activity [^{153}Sm]EDTA and [^{67}Ga]citrate (10.9 nmol kg^{-1} whole body) were injected i.v. into the surgically-exposed femoral vein of Copenhagen × Fisher rats bearing transplanted Dunning R3327-AT tumors (100 mm mean diameter). Scintigrams were recorded at 15 and 30 min, and 1, 6, 12, 24, and 48 hr after dosing. Animals dosed with [^{153}Sm]EDTA showed blood-pool and kidney images after 1 hr, but by 6 hr, especially in the animals which had voided, tumor, kidney, liver and bone were clearly delineated, whereas a scintigram of comparable quality was not seen after injection of ^{67}Ga. The 12-, 24-, and 48-hr scintigrams provided a clear view of the tumors with virtually no blood or intestinal background (Fig. 1).

Whole-body retention of both low and high specific activity [^{153}Sm]EDTA and [^{67}Ga]citrate was measured over a 48-hr period. At each specific activity, retention of ^{67}Ga was approximately double that of ^{153}Sm (Table 3). Fecal radioactivity accounted for 1 ± 0.5% and urine contained 80 ± 4% of the ^{153}Sm dose after 48 hr. Clearance of ^{153}Sm from the blood occurred with an overall half-life of < 5 min (Table 4); preliminary analysis of these data using graphical methods suggested a complex, perhaps triexponential, clearance pattern.

Toxicity studies in mice (15 ± 1 g) dosed with i.v. Sm-EDTA (0.1, 1.09 or 10.9 μmol Sm kg^{-1}) or i.v. normal saline, failed to reveal any significant ($p < 0.05$; n = 4 per group) change in weight gain over a 64-day observation period. During this time, the mice gained 16 ± 2 g. Histopathologic examination of lung, liver, kidney, and long bone (femur) detected no major his-

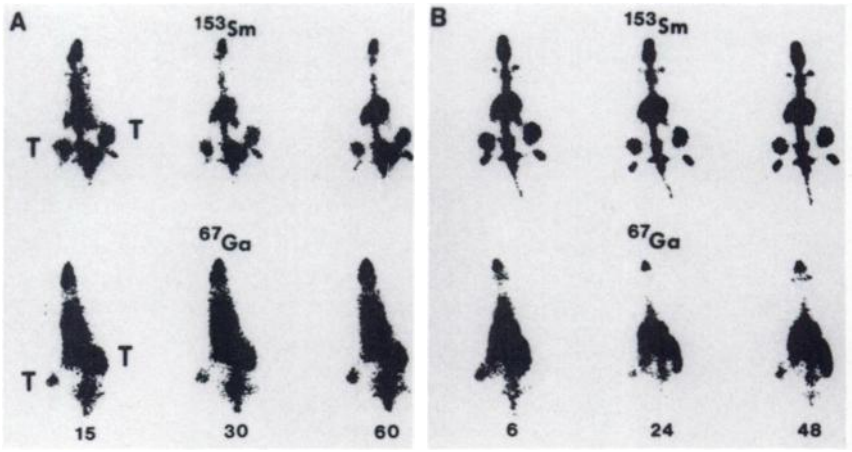

FIGURE 1
Whole-body scintigrams of Fisher × Copenhagen rats bearing transplanted Dunning R3327-H prostatic tumors. Samarium-153 EDTA (upper row) and [^{67}Ga]citrate (lower row) were administered at a radionuclide dose of 10.9 nmol kg^{-1} body weight. Scintigrams were recorded at (A) 15, 30, and 60 min, and (B) 6, 24, and 48 hr after i.v. injection. T indicates the location of the bilaterally-implanted tumors.

tologic changes over a 64-day period, nor in the first generation male offspring of female mice dosed at 1.09 μmol Sm kg^{-1} body weight. A nonsignificant number of hyaline casts were observed in the proximal tubules 24 hr after injection of the two higher dose ranges, along with a slight increase in the number of bi- and trinucleated regenerating liver cells.

DISCUSSION

Interest in the development of effective, low cost, diagnostic radiopharmaceuticals which have widespread geographic availability has led to a number of studies of reactor-produced radionuclides, including ^{153}Sm. In the present studies, $^{153}Sm_2O_3$ was produced in both low and high thermal neutron flux (1×10^{12} and 2×10^{14} n·cm^{-2}·sec^{-1}, respectively) research reactors. Yields, specific activity, and radionuclidic purity were similar to published data (*17,19*). Preparation of the EDTA chelate, from the $^{153}SmCl_3$ intermediate, has been described elsewhere for other ^{153}Sm complexes (*4,11–15*). As a note of caution, however, it was found to be essential that neutralization of the [^{153}Sm]Cl_3EDTA solution to pH 6.5 be approached slowly and with care to avoid high local concentrations of base which may cause formation of hydroxides/oxides, thereby resulting in some colloid formation. Traces of solids could be removed by filtration through a 0.22-micron filter, without appreciable losses of radioactivity.

When formulated with a tenfold molar excess of Na_2EDTA, the [^{153}Sm]EDTA was stable in both RPMI and Waymouth growth media and in the presence of cultured cells. At a twofold EDTA excess, chromatography indicated that ~ 50% of the ^{153}Sm was chelated, whereas only chelated ^{153}Sm was detected at the 1:10 ratio. Similar observations on the stability of ^{153}Sm complexes have been reported for citrates and other ligands (*13,15*). The chemical nature of the [^{153}Sm]EDTA complex has not been reported, but with nine coordination numbers, it has been pointed out that $Sm(EDTA)^-$, $Sm(EDTA)_2^{5-}$ and $Sm_2(EDTA)_3^{6-}$ are possible and at least two species have been detected by

TABLE 3
Whole-Body Retention of Low- and High Specific Activity [^{153}Sm]EDTA and [^{67}Ga] Citrate after i.v. Injection into Fisher x Copenhagen Rats Bearing Transplanted Dunning R3327-H Prostatic Tumors

Time after injection (hr)	% of Radioactivity Remaining in Body			
	Low Specific Activity*		High Specific Activity†	
	^{153}Sm	^{67}Ga	^{153}Sm	^{67}Ga
1	100‡	98.9 ± 0.5	95.6 ± 0.5	99.6 ± 0.3
6	18.9 ± 2.5§	83.6 ± 1.9	47.0 ± 0.9	81.6 ± 0.9
12	18.8 ± 2.1	74.3 ± 3.1	42.5 ± 1.1	75.2 ± 2.4
24	19.2 ± 2.7	59.4 ± 0.8	36.5 ± 0.9	57.2 ± 0.9
	19.2 ± 4.4	44.7 ± 0.3	23.7 ± 0.5	40.5 ± 0.4

* 1.09 umol radionuclide kg^{-1}.
† 10.9 nmol radionuclide kg^{-1}.
‡ not voided.
§ mean ± s.d., n = 3.

TABLE 4
In Vivo Blood Clearance of ^{153}Sm after i.v. Injection of [^{153}Sm]EDTA (1.09 umole Sm kg^{-1}) into Copenhagen x Fisher Rats Bearing Subcutaneously Transplanted Dunning R-3327H Prostatic Tumors

Time (min) After Injection	% of Injected Radioactivity Remaining in Blood*
1	86.0 ± 4.4
5	32.6 ± 8.6
10	18.0 ± 1.4
15	16.1 ± 0.9
20	12.6 ± 3.0
25	9.9 ± 0.9
30	8.8 ± 0.5
45	5.9 ± 0.4
60	3.5 ± 0.8
120	1.9 ± 0.8

* Mean ± s.d., n = 3.

high performance liquid chromatography (HPLC) when $^{153}SmCl_3$ was added to EDTA in a molar ratio of 1:10 (*19*). Although the 1:2 and 1:10 formulations produced similar scintigraphic distribution patterns in rats at early time periods, by ~ 6 hr after injection a slow, absolute increase in hepatic radioactivity was observed with the 1:2 formulation. This suggests that colloid was gradually being formed in vivo and that radioactivity was redistributed to the reticuloendothelial system without further excretion from the body.

Uptake of [^{153}Sm]EDTA at low specific activities in cell culture was quantitatively similar to uptakes of [^{67}Ga]citrate at similar molar concentrations. In the dual-labeled [^{153}Sm][^{14}C]EDTA study, the ratio of ^{153}Sm:^{14}C uptake was virtually constant in human Melanoma 2AB cells (2.29 ± 0.21 vs. 1.99 ± 0.06%). At high specific activity, there were no significant changes in ^{67}Ga or [^{14}C]EDTA uptakes by murine EMT-6 cells, but [^{153}Sm] uptake increased by four times the low specific activity values, to 20.7 ± 0.9%. The reason for this response may reflect differences between the murine and human cells used, or simply the limited uptake capacity which can transport or bind a larger fraction of the smaller dose.

The biodistribution of both low or high specific activity [^{153}Sm]EDTA in Lewis lung carcinoma-bearing BDF_1 mice was characterized by low blood levels (1.6–0.006% I.D. g^{-1}) and low uptake by tumor (1.95–0.39% I.D., g^{-1}), with slightly higher values for the high specific activity formulation (Table 2). In liver and kidney, the latter formulation produced two to three times higher levels at all periods except at 24 and 48 hr, when specific activity did not effect [^{153}Sm] content. Carbon-14 EDTA from doubly-labeled [^{153}Sm][^{14}C]EDTA was concentrated to only 10–20% of the [^{153}Sm] concentration in liver, which represents the stoichiometric ratio (1:10) of the formulation. In bone [^{153}Sm] concentrations are in the range of 20–30 times greater than ^{14}C. However, this difference was greatly reduced in tumor and kidney, and in fact was reversed in blood, where [^{14}C]EDTA % I.D. g^{-1} levels were double those of [^{153}Sm]. In general, these data support the concept that [^{153}Sm]EDTA is taken up by kidney and tumor, but that other forms, possibly colloids, are being deposited in RES-rich tissues such as liver and bone (marrow). Overall, the [^{153}Sm]EDTA tumor uptake (% I.D. g^{-1}) was lower than for other ^{153}Sm chelates ([^{153}Sm]HIDA 5.2–2.7 and 5.2–1.4% I.D. g^{-1}; [^{153}Sm]HEDTA 5.3–2% and 3.5–2.1% I.D. g^{-1}; and [^{153}Sm]diethylenetriaminepentaacetic acid 3.8–0.3 and 2.4–0.4% I.D. g^{-1}, respectively, for melanotic and amelanotic B16 melanomas in C57 black mice) (*13*) or for the $B_6D_2F_1$ Lewis lung model after injection of [^{153}Sm]citrate (5.8–10.7% I.D. g^{-1}) (*15*). Very low blood levels, however, compensate in part for this lower uptake when imaging is the final objective.

The optimal tumor indices (% I.D. g^{-1} × T.B.) (*20*) for low and high specific activity [^{153}Sm]EDTA were 37 (6 hr) and 51 (48 hr), respectively, compared with 302 (24 and 48 hr) for [^{153}Sm]citrate in this tumor model. Tumor indices for [^{67}Ga] citrate at low and high specific activities were 16 (24 hr) and 4.5 (48 hr), respectively, compared with 48.5 (24 hr) for an even lower specific activity product (22 μmol Ga kg^{-1} body weight) in this tumor model.

Whole-body clearance studies in Copenhagen × Fisher rats bearing transplanted Dunning R3327-H prostatic tumors confirmed that low specific activity [^{153}Sm]EDTA was rapidly excreted via the urine, and that within a few hours after injection, excretion virtually ceased. In contrast, excretion of the high specific activity chelate was progressive throughout the 48-hr observation period, although the first 6 hr still accounted for over half of the injected dose (Table 3). Total 48-hr excretion was similar (19.2 ± 4.4 vs. 23.7 ± 0.5) for the two formulations. The time course of whole-body elimination was not reflective of blood clearance. The blood clearance profile, when analyzed by graphic approximation as a triexponential function, indicated that the slow clearance represented < 15% of the dose, with a half-time of ~ 1 hr. The intermediate component represented 15–20% of the dose with an estimated half-time of 10 min, and the rapid component accounted for the remainder of the dose (65–70%) which was cleared from the blood with a half-time of 1–2 min. In large part these data represent dilution and distribution (short $T_{½}$), urinary excretion (intermediate $T_{½}$) and redistribution (long $T_{½}$) processes. Fecal excretion, at ~ 1% I.D. per 48 hr, plays a negligible role in either blood clearance or whole-body elimination despite substantial hepatic uptake.

Scintigraphic studies with the low and high specific activity [^{153}Sm]EDTA and [^{67}Ga]citrate formulations clearly demonstrated the superiority of scintigrams obtained with high specific activity [^{153}Sm]EDTA over scintigrams from other products. Quantitative biodistribution measurements were not made for the rat model, but it is evident that rapid and almost total ^{153}Sm blood clearance and adequate tumor uptake resulted in clear delineation of the tumor mass from other soft tissues. Although ^{153}Sm was present in liver and the skeleton, the [^{153}Sm]EDTA images were superior to [^{67}Ga]citrate even in these areas because of the absence of blood background and intestinal radioactivity.

The absence of observable histopathologic effects after relatively large doses of [^{153}Sm]EDTA (up to 10.9 μmol kg^{-1} vs. 10.9 nmol kg^{-1} for imaging) in preliminary studies, along with the observed biodistribution and excretion patterns in mice and rats, are supportive of the clinical application of [^{153}Sm]EDTA. With good tumor delineation within 6 hr and a shorter $T_{½}$ compared to ^{67}Ga, absorbed radiation doses from this radi-

otracer are expected to be ~ 20% higher than for [^{67}Ga] citrate, which requires a 48–72 hr wait after injection and has a 50% longer physical $T_{½}$. Although no attempt has been made to provide detailed dose calculations, biodistribution data and elimination half-time estimates of 57, 34, and 27 hr (liver, bone, and kidney, respectively) suggest doses of 0.4, 0.09, and 0.3 mGy MBq^{-1} to the respective organs. Recently published data (*21*) for [^{153}Sm]EDTMP, a bone-seeking radiotherapeutic agent, list doses of 0.028, 1.03, and 0.108, mGy MBq^{-1}, respectively, for liver, bone marrow and kidney.

In summary, [^{153}Sm]EDTA formulated with a tenfold excess of Na_2EDTA at a final pH of 6.5 is sufficiently stable for use as a radiotracer in vitro and in vivo. At lower specific activity (14.3 GBq $mmol^{-1}$), [^{153}Sm] EDTA showed a greater tendency for liver and bone uptake than at higher specific activity (22.1 TBq mmol $^{-1}$Sm). With high specific activity ^{153}Sm, it was possible to reduce by two orders of magnitude (e.g., to 1%) the mass of Sm injected. This presumably reduced the formation of colloid in vivo and at the same time improved uptake (as % I.D. g^{-1}) by the tumor. Excellent tumor delineation, low acute and chronic toxicity, and the potential for "sameday" dosing and imaging, provide a rationale for further investigation of high specific activity [^{153}Sm]EDTA for diagnostic tumor scintigraphy.

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