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Localization of Small-cell Lung Cancer Xenografts with Iodine-125-, Indium-111-, and Rhenium-188-Somatostatin Analogs

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We examined the potential of radiolabeled somatostatin analogs, ^{125}I-Tyr-3-octreotide (^{125}I-octreotide), ^{111}In-DTPA(diethylenetriaminepentaacetatic acid)-D-Phe-1-octreotide (^{111}In-octreotide), and ^{188}Re-octreotide for targeting small-cell lung cancer (SCLC) in a mouse model. Tyr-3-octreotide was labeled with ^{125}I by the chloramine T method, and ^{111}In-octreotide was obtained as a kit, while ^{188}Re was eluted from a ^{188}W/^{188}Re generator, and octreotide was directly labeled with ^{188}Re by reducing disulfide bonds. The ^{125}I-, ^{111}In-, and ^{188}Re-octreotides were injected i.v. into athymic mice bearing NCI-H69 tumors, and the biodistributions were determined at 15 min, and 2, 4, 8, and 24 h. Tumor uptakes were 0.5±0.2, 0.3±0.1, 0.3±0.1 %ID/g, and tumor-to-blood ratios were 1.8, 11.9, 1.2 at 8 h for ^{125}I-, ^{111}In-, and ^{188}Re-octreotides, respectively. Accumulations of ^{111}In-octreotide in normal tissues were lower than those of ^{125}I- and ^{188}Re-octreotides. ^{188}Re-octreotide can be used to localize SCLC lesions as efficiently as radioiodinated octreotide. However, ^{111}In-octreotide was the most suitable agent to obtain high tumor-to-normal tissue contrast for localizing SCLC.

Key words: Small-cell lung cancer — Octreotide — Rhenium-188 — Xenograft

Small-cell lung cancer (SCLC), characterized by endocrine features, a tendency to metastasize, and high chemo- and radiosensitivity, accounts for 20–25% of lung cancers.[1)] Because most SCLC tumors have spread at the time of diagnosis, targeting lesions with radiopharmaceuticals would be useful to evaluate tumor extension and also to treat tumors with metastases. Laboratory and clinical studies have suggested the usefulness of radiolabeled monoclonal antibodies (mAbs) for the diagnosis and therapy of SCLC tumors.[2–6)] On the other hand, as SCLC tumors may synthesize somatostatin and most of them have high-affinity somatostatin-binding sites,[7–9)] the possibility of using radiolabeled somatostatin analogs has also been examined in animal models and humans. Kwekkeboom *et al.* reported that in the majority of their patients with SCLC, the tumor and its metastases could be visualized with a radiolabeled somatostatin analog ^{123}I-Tyr-3-octreotide,[10)] and O'Byrne *et al.* demonstrated that ^{111}In-DTPA (diethylenetriaminepentaacetatic acid)-D-Phe-1-octreotide facilitated the clinical evaluation of patients with SCLC.[11)] Some authors have suggested that ^{111}In-DTPA-D-Phe-1-octreotide is more suitable for localizing somatostatin receptor-rich tissues than ^{123}I-Tyr-3-octreotide because of lower background in the circulation and the lower hepatobiliary and intestinal accumulations.[12)]

^{188}Re, readily obtained from a ^{188}W/^{188}Re generator, has a gamma energy suitable for imaging and a beta energy appropriate for internal radiotherapy. Recently, we have determined the feasibility of direct labeling of octreotide with ^{188}Re by reducing disulfide bonds between the cysteine residues.[13, 14)] The purpose of this study was to examine and compare the abilities of ^{125}I-Tyr-3-octreotide (^{125}I-octreotide), ^{111}In-DTPA-D-Phe-1-octreotide (^{111}In-octreotide), and ^{188}Re-labeled octreotide (^{188}Re-octreotide) to localize SCLC xenografts.

MATERIALS AND METHODS

Radiolabeling of peptides Tyr-3-octreotide, obtained from Sandoz (Basel, Switzerland), was labeled with ^{125}I using chloramine T as reported previously by Bakker *et al.*[15)] Briefly, 10 μg of Tyr-3-octreotide in 80 μl of phosphate buffer (PB, 0.3 *M*, pH 7.5) was mixed with 37 MBq of ^{125}I (Amersham, Buckinghamshire, UK) and 10 μg of chloramine T in 10 μl of PB. After incubation for 4 min, the iodination was stopped by addition of 1 ml of PB. Then, ^{125}I-labeled Tyr-3-octreotide was purified using a SEP-PAK C18 mini-column (Millipore Inc., Bedford, MA). After application of the ^{125}I-Tyr-3-octreotide solu-

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tion, the column was washed with 5 ml of distilled water, and radioiodinated Tyr-3-octreotide was eluted with 5 ml of 85% ethanol.

Kits of ^{111}In-DTPA-D-Phe-1-octreotide were provided as Octreoscan (Mallinckrodt Medical, Petten, The Netherlands). The labeling was conducted according to the manufacturer's instructions. The ^{111}In-octreotide was used for *in vitro* and *in vivo* studies without further purification.

Perrhenate solution was obtained from a ^{188}W/^{188}Re generator supplied by Oak Ridge National Laboratory (Oak Ridge, TN). Octreotide, purchased as Sandostatin 500 (Sandoz), was labeled with ^{188}Re as previously reported.[13, 14] Briefly, octreotide was dissolved in nitrogen-purged water to a concentration of 500 μg/ml. An equal volume of solution containing 20 m*M* tartrate, 80 m*M* phthalate, 4% maltose, 100 m*M* glycine, and 2 m*M* stannous chloride (pH 5.6) was added to the octreotide solution. The mixed solution was aliquoted (2 ml) into vials. The vials were then sealed under an atmosphere of nitrogen, allowed to incubate for 4 h at room temperature, and stored frozen at -30°C until use. For labeling, a vial was thawed to room temperature and 370 MBq (10 mCi) in a 2 ml perrhenate solution from the ^{188}W/^{188}Re generator was added. The solution was heated in a water bath at 100°C for 30 min. The ^{188}Re-labeled octreotide was used for *in vitro* and *in vivo* studies without further purification.

The radiolabeled octreotide solutions were diluted with 10% human serum albumin (Immuno GmbH, Heidelberg, Germany).

Quality control of radiolabeled peptides Thin layer chromatography was used to determine the labeling efficiency and colloid formation of peptides. ITLC-SG chromatography paper #61886 (Gelman Sciences, Ann Arbor, MI) was cut into 1.5×10 cm strips and activated by heating for 30 min at 110°C according to the manufacturer's instructions. The strips were stored dry at room temperature until use. Labeling efficiency was determined using 0.9% saline as a mobile phase. The unbound radioactivity migrated with the solvent front, while the bound radioactivity remained at the origin. The amount of colloids formed in the peptide solution was determined using 85% ethanol as a mobile phase. The radiolabeled peptides moved to the solvent front and colloids remained at the origin.

After purification on a SEP-PAK C18 mini-column, ^{125}I-octreotide was analyzed by reversed-phase, high-performance liquid chromatography (HPLC) using a LiChrosorb RP-18 column, 250×4 mm, particle size 7 μm (VDS Optilab Chromatographietechnik GmbH, Berlin, Germany). A gradient of 0% to 60% acetanilide in 0.05 *M* triethylammonium phosphate (pH 2.25) was applied over 30 min.

Cell line and xenografts The SCLC cell line NCI-H69[16] was cultured in RPMI 1640 culture medium (Gibco, Grand Island, NY) supplemented with 1 m*M* glutamine and 10% fetal calf serum. For studies in mice, 5- to 7-week-old female NMRI *nu/nu* athymic mice were purchased from Harlan (Borchen, Germany). NCI-H69 SCLC cells (1×10^7 per mouse) were implanted by s.c. inoculation into the flanks of mice. Xenografts were allowed to grow 3 to 4 weeks after inoculation. The weight of xenografts used in this study ($n=72$) was 0.25 $\pm$0.14 g and there was no significant difference among groups. The thyroids of the mice were not blocked. All animal studies were conducted in Germany under an approved animal protocol.

Cell binding assay To determine the binding to cells, ^{125}I-, ^{111}In-, and ^{188}Re-octreotides diluted with 10% human serum albumin (Immuno GmbH, Heidelberg, Germany) in saline (5 ng/100 μl) were incubated with increasing concentrations of NCI-H69 cells in 5.7×46 mm microcentrifuge tubes for 1 h at 4°C. After centrifugation at 1,500*g*, the tubes were washed with saline and cut. The radioactivity bound to cells was counted in a well-type gamma counter. Specific binding to cells was calculated by subtracting the nonspecific binding in tubes to which 5 μg of unlabeled octreotide had been added.

Biodistribution of radiolabeled peptides Groups of 4 mice bearing NCI-H69 xenografts per time point were given 37 kBq of ^{125}I-octreotide, 37 kBq of ^{111}In-octreotide, or 185 kBq of ^{188}Re-octreotide via the tail vein. At 15 min, 2, 4, 8, and 24 h after injection, the mice were killed. Tumors and selected organs were weighed and the radioactivity was determined with a well-type gamma counter.

In order to saturate somatostatin receptors on the tumors and normal tissues in 3 groups of mice, unlabeled octreotide (500 μg) was injected s.c. 1 h prior to the administration of ^{125}I-, ^{111}In-, and ^{188}Re-octreotides, and biodistributions were determined at 2 h after the injection of radiolabeled peptides. The results were expressed as the percentage of the injected dose per gram of tissue (%ID/g). Data were analyzed by using Student's *t* test. A P value of <0.05 was considered significant.

RESULTS

The labeling efficiency was 70%, >97%, and >99% for ^{125}I-, ^{111}In-, and ^{188}Re-octreotides, respectively, as determined by ITLC with 0.9% saline as the mobile phase. The amount of radiocolloid determined by ITLC with 85% ethanol as the mobile phase was less than 3% for all of them. After purification on a SEP-PAK C18 mini-column, the ^{125}I-octreotide solution was subjected to reversed-phase HPLC. Monoiodinated octreotide was eluted at the retention time of 16.7 min, while

deiodinated octreotide was eluted at 18 min, indicating that monoiodinated octreotide accounted for more than 95% of the radioactivity.

As illustrated in Fig. 1, the *in vitro* binding of ^{188}Re-octreotide to NCI-H69 cells was similar to that of ^{125}I-octreotide, while ^{111}In-octreotide showed lower binding to the cells.

In mice, all three radiolabeled octreotides were rapidly taken up by NCI-H69 tumors, resulting in the maximal tumor uptake at 15 min (Tables I, II, and III). From 2 to 24 h, the tumor uptakes of ^{125}I- and ^{188}Re-octreotides were significantly higher than that of ^{111}In-octreotide, which cleared from the circulation faster than ^{125}I- and ^{188}Re-octreotides. A substantial amount of radioactivity was observed in the gastrointestinal (GI) tract from 15 min for ^{125}I- and ^{188}Re-octreotides. On the other hand, ^{111}In-octreotide accumulated in the kidney to a greater extent than ^{125}I- and ^{188}Re-octreotides localized in the GI tract or in the kidney. After the time point of 2 h, accumulations in the liver, kidney and GI tract were higher than in the blood for all radiolabeled octreotides. Presaturation with unlabeled octreotide led to a significant decrease of radioactivity in the xenografts for ^{125}I-, ^{111}In-, and ^{188}Re-octreotides (Table IV). Fig. 2 shows the tumor-to-blood ratios. Tumor-to-blood ratios were 0.91, 7.45, 0.41 at 2 h, 1.77, 11.86, 1.23 at 8 h for ^{125}I-, ^{111}In-, and ^{188}Re-octreotides, respectively. At 8 h, tumor-to-liver ratios were 0.69, 2.11, 0.11, and tumor-to-large intestine ratios were 0.22, 0.82, 0.24 for ^{125}I-, ^{111}In-, and ^{188}Re-octreotides, respectively. Tumor-to-normal tissue ratios of ^{111}In-octreotide were significantly elevated as compared to those of ^{125}I- and ^{188}Re-octreotides, with the exception of the tumor-to-kidney ratios.

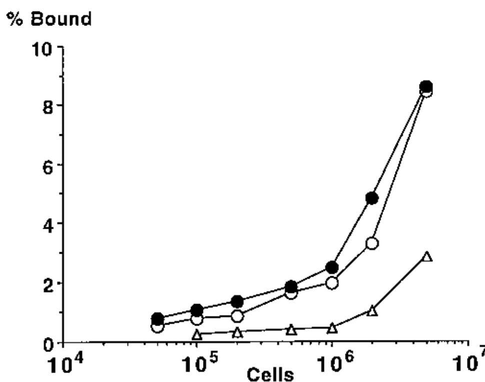

Fig. 1. Cell-binding curves of ^{125}I-octreotide (open circles), ^{111}In-octreotide (open triangles), and ^{188}Re-octreotide (closed circles). The vertical axis indicates percentage of bound radioactivity to total radioactivity. Nonspecific binding was subtracted.

DISCUSSION

It has been reported that most SCLC tumors have high-affinity somatostatin binding sites, and the xenograft tissue of NCI-H69 expresses somatostatin receptor homogenously at high density.[7-9] We confirmed the expression of somatostatin receptor on NCI-H69 cells by a cell-binding assay (Fig. 1), in which ^{125}I- and ^{188}Re-octreotides showed similar binding to NCI-H69 cells, while ^{111}In-octreotide demonstrated lower binding. This may be due to the lower affinity of ^{111}In-DTPA-D-Phe-1-octreotide to the somatostatin receptor, as found by Bakker *et al.*[17]

In this study, we evaluated the effectiveness of radiolabeled octreotides for the localization of SCLC xenografts. Absolute accumulation levels in xenografts of

Table I. Biodistribution (%ID/g) of ^{125}I-Labeled Tyr-3-octreotide in Athymic Mice Bearing NCI-H 69 Tumors (mean±1 SD).

Organ	Time after injection				
	15 min	2 h	4 h	8 h	24 h
Blood	3.61±0.41	1.73±0.59	0.73±0.16	0.30±0.13	0.09±0.01
Liver	13.78±0.75	5.18±0.89	1.80±0.06	0.76±0.13	0.32±0.04
Kidney	11.13±1.26	4.93±0.35	2.83±0.42	1.38±0.24	0.49±0.10
Stomach	5.36±3.18	7.57±2.48	4.46±0.82	1.29±1.38	0.23±0.11
SI	17.30±4.23	9.45±5.32	1.47±0.46	0.37±0.11	0.08±0.04
LI	1.04±0.28	20.62±8.20	29.20±12.72	2.45±0.62	0.26±0.15
Spleen	1.43±0.22	0.88±0.15	0.48±0.07	0.19±0.06	0.09±0.00
Lung	4.07±0.41	1.73±0.29	0.98±0.08	0.45±0.07	0.24±0.03
Brain	0.13±0.02	0.08±0.02	0.06±0.02	0.01±0.01	0.01±0.00
Femur	1.13±0.58	0.51±0.13	0.57±0.39	0.11±0.06	0.03±0.01
Tumor	2.08±0.69	1.47±0.40	0.88±0.30	0.52±0.21	0.27±0.04

SI, small intestine; LI, large intestine.

Table II. Biodistribution (%ID/g) of ^{111}In-Labeled DTPA-D-Phe-1-octreotide in Athymic Mice Bearing NCI-H69 Tumors (mean±1 SD).

Organ	Time after injection				
	15 min	2 h	4 h	8 h	24 h
Blood	2.92±0.32	0.06±0.01	0.03±0.00	0.02±0.00	0.01±0.00
Liver	1.26±0.06	0.23±0.02	0.16±0.01	0.12±0.01	0.04±0.00
Kidney	30.30±4.89	19.42±2.07	12.98±3.93	12.84±2.68	2.93±0.39
Stomach	0.74±0.19	0.53±0.21	0.25±0.09	0.18±0.12	0.12±0.04
SI	0.90±0.22	0.41±0.11	0.12±0.03	0.13±0.05	0.06±0.02
LI	1.60±1.32	0.49±0.08	0.79±0.28	0.33±0.11	0.25±0.10
Spleen	0.74±0.19	0.10±0.01	0.09±0.02	0.07±0.01	0.04±0.02
Lung	2.38±0.58	0.18±0.02	0.09±0.01	0.09±0.04	0.03±0.01
Brain	0.10±0.03	0.02±0.01	0.01±0.01	0.01±0.00	0.01±0.00
Femur	1.95±2.13	0.10±0.02	0.18±0.11	0.04±0.02	0.04±0.03
Tumor	2.61±0.51	0.43±0.13	0.28±0.06	0.26±0.08	0.12±0.02

SI, small intestine; LI, large intestine.

Table III. Biodistribution (%ID/g) of ^{188}Re-Labeled Octreotide in Athymic Mice Bearing NCI-H69 Tumors (mean±1 SD).

Organ	Time after injection				
	15 min	2 h	4 h	8 h	24 h
Blood	3.93±0.95	1.16±0.21	0.50±0.06	0.27±0.10	0.09±0.02
Liver	14.93±1.82	8.97±1.21	5.32±0.27	3.36±0.53	1.42±0.26
Kidney	11.41±3.69	19.57±2.67	13.99±5.13	11.29±3.14	2.90±0.78
Stomach	5.21±5.04	3.56±1.97	1.86±0.68	0.68±0.42	0.31±0.12
SI	13.81±5.64	5.76±3.10	1.65±0.95	0.54±0.15	0.19±0.02
LI	0.55±0.17	14.07±2.21	13.74±12.11	1.44±0.49	0.63±0.15
Spleen	2.95±0.60	1.97±0.58	1.47±0.45	1.35±0.13	0.67±0.25
Lung	4.36±0.65	2.16±0.26	1.83±0.45	1.35±0.21	0.54±0.17
Brain	0.11±0.03	0.04±0.01	0.02±0.01	0.00±0.00	0.04±0.02
Femur	0.83±0.29	0.84±0.21	0.39±0.07	0.30±0.05	0.19±0.10
Tumor	0.68±0.18	0.46±0.05	0.42±0.12	0.34±0.08	0.18±0.02

SI, small intestine; LI, large intestine.

Table IV. Accumulation of ^{125}I-, ^{111}I-, and ^{188}Re-Octreotides with and without Pretreatment of Unlabeled Octreotide

Tissue		^{125}I-octreotide	^{111}In-octreotide	^{188}Re-octreotide
Tumor	−	1.47±0.40	0.43±0.13	0.46±0.05
	+	0.84±0.21*	0.28±0.08*	0.18±0.03*
Pancreas	−	1.65±0.34	0.61±0.09	0.70±0.10
	+	0.62±0.29*	0.06±0.01*	0.41±0.21*
Kidney	−	4.93±0.35	19.42±2.07	19.57±2.67
	+	2.37±0.32*	11.59±3.30*	9.18±0.94*

−, +; Without and with unlabeled octreotide pretreatment.
* $P<0.05$ versus groups without pretreatment.

^{111}In-octreotide were lower than those of ^{125}I- and ^{188}Re-octreotides from 2 to 24 h. However, tumor-to-normal tissue ratios of ^{111}In-octreotide became higher than those of ^{125}I- and ^{188}Re-octreotides except for tumor-to-kidney ratios, because ^{111}In-octreotide was cleared more rapidly from the circulation. The time points of 2 to 24 h seemed to be favorable for high tumor-to-blood contrast (Fig. 2). Elevated tumor-to-normal tissue ratios are advantageous for imaging as they provide clearer images. Mice administered with ^{111}In-octreotide would be exposed to less whole-body radiation than mice administered with ^{125}I- or ^{188}Re-octreotide for a given dose delivered to the tumor.

Our results for ^{111}In-octreotide are in agreement with a previous report by Manil *et al.*[18] They noted tumor-to-liver ratios of 0.9–6.2 at 24 h after injection of ^{111}In-octreotide in mice bearing human neuroblastoma or small-cell lung cancer tumors.

In comparing ^{125}I-octreotide with ^{188}Re-octreotide, there may be no practical difference between the two for

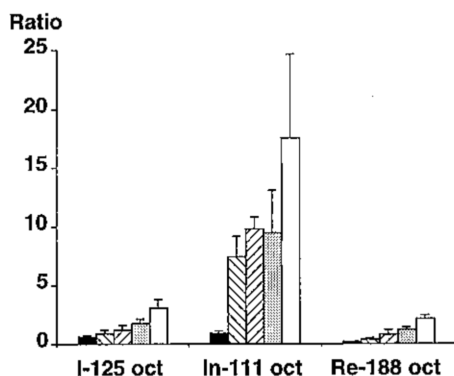

Fig. 2. Tumor-to-blood ratios of ^{125}I-Tyr-3-octreotide (I-125 oct), ^{111}In-DTPA-D-Phe-1-octreotide (In-111 oct), and ^{188}Re-octreotide (Re-188 oct) in mice xenografted with NCI-H69 at 15 min (■), 2 (▧), 4 (▨), 8 (▩), and 24 h (□).

detecting abdominal lesions because of the similar tumor-to-blood and tumor-to-large intestine ratios. The hepatobiliary clearance of ^{125}I- and ^{188}Re-octreotides results in high hepatic and intestinal accumulation of radioactivity, which disturbs scintigraphic imaging of the abdomen.

The maximum beta energy of ^{188}Re, 2.1 MeV, is suitable for the therapy of tumors, which are often heterogeneously necrotic. The peak gamma energy of ^{188}Re, 155 keV, is suitable for imaging. Eary *et al.* suggested that a medium-energy collimator is appropriate to get optimal image resolution and count rate for ^{188}Re because of its higher energy peaks, including 478, 633, 829, and 931 keV.[19] Although ^{186}Re has been considered as a radionuclide for labeling with mAbs and peptides, it is usually produced by neutron exposure of a ^{185}Re-rich metal target, and it is difficult to get high-purity ^{186}Re. Therefore, ^{188}Re seems more relevant for routine labeling of antibodies and peptides than ^{186}Re.

The detection of adrenal metastases, often observed in patients with lung cancer, is difficult with ^{111}In-octreotide due to its substantial accumulation in the kidney. However, Hammond *et al.* reported that renal tubular uptake of ^{111}In-octreotide could be reduced by i.v. amino acid infusion.[20] This may facilitate diagnosis and internal radiotherapy using radiolabeled octreotide by decreasing the renal absorption irradiation dose.

Immunoconjugates have been used to target SCLC.[2–6] Hosono *et al.* reported that mAb NE150, recognizing neural cell adhesion molecule, localized NCI-H69 tumors in an athymic mouse model. ^{125}I- and ^{111}In-labeled NE150 showed tumor uptakes of 12.1 and 13.9%ID/g and tumor-to-blood ratios of 0.74 and 0.98, respectively, at 24 h.[5] In the present study, the tumor uptakes of ^{125}I- and ^{111}In-octreotides were 0.14 and 0.11%ID/g, and tumor-to-blood ratios were 1.6 and 16.9, respectively, at 24 h. Therefore, it is most probable that radiolabeled octreotides offer higher tumor-to-blood contrast than radiolabeled intact IgG. Moreover, radiolabeled octreotide has the advantage of the absence of HAMA response, which murine mAbs cause even if they are chimerized.[21]

In conclusion, radiolabeled octreotides seem to be suitable for targeting SCLC tumors. ^{111}In-octreotide localizes SCLC efficiently except for renal and adrenal lesions. Radioiodinated Tyr-3-octreotide and ^{188}Re-octreotide can contribute to the detection of SCLC lesions, but less effectively than ^{111}In-octreotide.

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