Biodistribution of ^{177}Lu-octreotate and ^{111}In-minigastrin in female nude mice transplanted with human medullary thyroid carcinoma GOT2

JOHANNA DALMO[1], NILS RUDQVIST[1], JOHAN SPETZ[1], PETER LAVERMAN[4], OLA NILSSON[3], HÅKAN AHLMAN[2] and EVA FORSSELL-ARONSSON[1]

Departments of [1]Radiation Physics, and [2]Surgery, Institute of Clinical Sciences, Sahlgrenska Cancer Center, Sahlgrenska Academy, University of Gothenburg, Gothenburg; [3]Department of Pathology, Institute of Biomedicine, Sahlgrenska Cancer Center, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [4]Department of Nuclear Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Received July 25, 2011; Accepted August 19, 2011

DOI: 10.3892/or.2011.1494

Abstract. To be able to evaluate new radiopharmaceuticals and optimize diagnostic and therapeutic procedures, relevant animal models are required. The aim of this study was to evaluate the medullary thyroid carcinoma GOT2 animal model by analyzing the biodistribution of ^{177}Lu-octreotate and ^{111}In-minigastrin (MG0). BALB/c nude mice, subcutaneously transplanted with GOT2, were intravenously injected with either ^{177}Lu-octreotate or ^{111}In-MG0, with or without excess of unlabeled human minigastrin simultaneously with ^{111}In-MG0. Animals were sacrificed 1-7 days after injection in the ^{177}Lu-octreotate study and 1 h after injection of ^{111}In-MG0. The activity concentrations in organs and tissues were determined and mean absorbed doses from ^{177}Lu were calculated. There was a specific tumor uptake of either ^{177}Lu-octreotate or ^{111}In-MG0. ^{177}Lu-octreotate samples showed high activity concentrations in tissues expressing somatostatin receptors (SSTR). For both radiopharmaceuticals the highest activity concentrations were found in the kidneys. Compared to results from similar studies in mice with another MTC cell line (TT) the biodistribution was favorable (higher tumor uptake) for the GOT2 model, while compared to other animal models expressing SSTR, the tumor uptake of ^{177}Lu-octreotate was modest. In conclusion, the GOT2 animal model is a valuable model for evaluation and optimization of diagnostic and therapeutic procedures using radiolabeled somatostatin, CCK2 and gastrin analogues prior to clinical studies.

Correspondence to: Johanna Dalmo, Department of Radiation Physics, University of Gothenburg, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden
E-mail: johanna.dalmo@radfys.gu.se

Key words: medullary thyroid carcinoma, ^{177}Lu-octreotate, biodistribution, CCK2 receptor, somatostatin receptor

Introduction

The recent advances in diagnostic imaging and radionuclide therapy of neuroendocrine (NE) tumors using radiolabeled somatostatin analogues have been prominent (1). The radiolabeled somatostatin analogue ^{111}In-[DTPA^0, D-Phe^1]-octreotide (^{111}In-octreotide) is routinely used for localization of somatostatin receptor (SSTR) expressing tumors of various types. ^{177}Lu-[DOTA^0, Tyr^3]-octreotate (^{177}Lu-octreotate) is used for therapy of carcinoid and endocrine pancreatic tumors with promising results. Much effort has been spent on finding other receptors overexpressed in tumors and radiolabeled receptor-binding peptides that could be used for radionuclide scintigraphy and therapy of other tumor types, e.g. cholecystokinin-2 (CCK2)/gastrin receptors expressed by medullary thyroid carcinoma (MTC).

To be able to study methods to enhance the therapeutic results and to compare the usefulness of new radiopharmaceuticals, relevant animal models are required for testing and optimization of the procedures. To enable translation of results to patients, the animal model should resemble the treatment situation as closely as possible, and include human tumors with retained properties relevant for therapy. Important issues are similar biodistribution and dosimetric data, tumor characteristics (tumor size and growth rate), radiation sensitivity, and similar normal tissue characteristics, including toxicity.

Today, there are few animal models available with peptide receptor-expressing human tumors. The GOT1 tumor cell line was derived from a human midgut carcinoid (2,3). The GOT1 tumor cells have been transplanted on nude mice and have well-preserved NE differentiation, express SSTR1-5 (mainly SSTR2 and 5) and have a slow growth rate (about 14-16 days doubling time). The low tumor growth rate can be an advantage, leading to more realistic therapeutic results. The NCI-H69 is a human small cell lung cancer cell line with rapid growth expressing SSTR (4,5). The BON cell line is a human pancreatic carcinoid cell line, expressing gastrin and somatostatin receptors, transplantable to nude mice, and with a

doubling time of about 13 days (6,7). KRJ-I is a human malignant ileal carcinoid cell line that has been characterized and confirmed to be of enterochromaffin (EC) cell type (8,9).

The TT cell line is a human MTC cell line, transplantable to nude mice, and expresses gastrin receptors and SSTR (10). For the study of MTC treatment strategies other models have been used, e.g. transfection of CCK2/gastrin receptors to human embryonic kidney cells (HEK293) or to human epidermoid carcinoma cells (A431) with subsequent transplantation to nude mice (11-13).

Numerous studies on radiolabeled octreotide, or octreotate, have been made on nude mice xenografted with GOT1 (14-17) or NCI-H69 (18-21). Studies using radioiodinated meta-iodobenzylguanidine (MIBG) have been performed *in vitro* or on nude mice xenografted with BON tumors (22,23).

Human MTC can have high expression of CCK2/gastrin receptors (24-26). These receptors can be targeted with gastrin analogues, e.g., Minigastrin-0 (MG0) (27). Targeting of CCK2/gastrin receptors with radiolabeled gastrin-like peptides may be a tool for radionuclide imaging and therapy, as previously studied in TT (28-31), HEK293 (32) and A431 cells/models (31) with different results.

Diagnostic imaging with gastrin-like peptides labeled with ^{111}In, or ^{99m}Tc, has previously been studied clinically with promising results (27,29,33-35). One study also examined the potential therapeutic effects of ^{90}Y-DTPA-MG0 leading to tumor stabilization at the cost of severe renal toxicity (27). Scintigraphic imaging studies of patients injected with ^{111}In labeled gastrin-like peptides, or octreotide, showed higher tumor detection rate using gastrin- than somatostatin-receptor images (94 vs. 41%) (35).

Since the access to human transplantable somatostatin and gastrin receptor expressing cell lines is limited, many researchers have used animal cell lines: the SSTR expressing rat pancreatic cell lines, AR42J and CA20948, from a hyperplastic exocrine pancreatic nodule and an acinar pancreatic tumor, respectively, after induction with azaserine (36-38).

The human MTC cells GOT2 can be xenotransplanted to nude mice (39) and have slow growth rate; an estimated doubling time of about 3 weeks. No biodistribution studies have to date been performed on GOT2 cells using radiolabeled somatostatin or CCK2/gastrin receptor targeting peptides.

The aim of this study was to evaluate the usefulness of the GOT2 animal model for evaluation of radiolabelled radiopharmaceuticals directed towards SSTR and CCK2/gastrin receptor by analyzing the biodistribution of ^{177}Lu-octreotate and ^{111}In-DOTA-MG0. The results were compared with similar data obtained in other animal models.

Materials and methods

Radiopharmaceuticals. ^{177}Lu-trichloride and [DOTA^0, Tyr^3]-octreotate were purchased from the Nuclear Research and consultancy Group (NRG, Petten, The Netherlands). Radiolabeling of [DOTA^0, Tyr^3]-octreotate with ^{177}Lu-trichloride was performed according to the instructions of the manufacturer. The fraction of peptide-bound ^{177}Lu was higher than 99%, as determined by instant thin layer chromatography (ITLC) with 0.1 M sodium citrate as the mobile phase.

DOTA-conjugated MG0 was obtained from the Department of Nuclear Medicine at Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. Human minigastrin (MG) was purchased from Sigma-Aldrich Sweden AB, Sweden. The DOTA-MG0 was radiolabeled with ^{111}In by incubation with $^{111}InCl_3$ (Mallinckrodt Medical B.V., Petten, The Netherlands) in 0.1 M ammonium acetate buffer, pH 5.5, for 30 min at 95˚C. The fraction of peptide-bound ^{111}In was higher than 95% according to ITLC.

GOT2 animal model. GOT2 tumor tissue samples were inoculated subcutaneously (s.c.) in the neck of female BALB/c nude mice (Charles River, Japan and Germany), aged 4-6 weeks, under anesthesia using 2.5% Avertin i.p. (Sigma-Aldrich Sweden AB). The age of the mice at start of experiments was 3-7 months; the tumor sizes were 30-615 mm^3 in the ^{177}Lu-octreotate and 100-1600 mm^3 in the ^{111}In-MG0 studies. Water and autoclaved food were available *ad libitum*. The studies were approved by the Ethics committee for Animal Research at University of Gothenburg, Göteborg, Sweden.

Biodistribution of ^{177}Lu-octreotate. GOT2 tumor-bearing nude mice were divided into seven groups injected with ^{177}Lu-octreotate; 5 MBq (3 groups, n=5/group), 10 MBq (3 groups, n=6/group), and 30 MBq (1 group, n=5/group), corresponding to 0.2, 0.4 and 1.3 μg peptide, respectively. The tumor size distribution within the groups was kept as similar as possible. All animals were injected intravenously into the tail vein. To determine the amount of radiopharmaceutical administered to each mouse, the syringes were measured before and after administration, using a well-type ionization chamber (CRC-15R, Capintec, NJ, USA), and values were corrected for detector background.

At day 1, 3 or 7 after injection, the mice were sacrificed by cardiac puncture under anesthesia with 2.5% Avertin (Sigma-Aldrich Sweden AB). Samples of blood and thigh muscle, together with the adrenals, spleen, kidneys, liver, heart, pancreas and tumor were collected. All tissue samples were weighed and the ^{177}Lu activity in each sample was measured using a Wallac 1480 γ counter (WIZARD™ 3'' Wallac Oy, Finland) with a 10% energy window over the 208 keV photon peak. Corrections for background and dead time were made. A calibration factor between the ionization chamber and the γ counter was determined and used.

Biodistribution of ^{111}In-MG0. Ten GOT2 tumor-bearing mice were divided into two groups (n=5/group). The mice were injected intravenously in the tail vein with 22 kBq of ^{111}In-DOTA-MG0 (^{111}In-MG0) in 0.15 ml solution. One group also received a 100-fold molar excess of unlabeled human minigastrin simultaneously.

All syringes containing ^{111}In-MG0 were drawn from the same batch solution and injected activity was determined by measurements on several 0.15-ml samples of the batch solution prior to injection using the γ counter described above. Correction for detector background was made.

The mice were sacrificed 1 h after injection as described above. The animals were immediately dissected and ^{111}In activity measurements were made *ex vivo* on adrenals, heart, kidneys, liver, lungs, spleen, tumor and samples of blood

(0.5-1 ml, from cardiac puncture) and muscle (from femur) using the γ counter.

Dosimetry. The ^{177}Lu and ^{111}In activity concentration in tissue at the time of injection, C_{tissue}, was calculated as the fraction of the injected activity, $A_{injected}$, per unit mass of the tissue:

$$C_{tissue} = \frac{A_{tissue} / m_{tissue}}{A_{inj}} \cdot 100\% \quad [\%IA/g]$$

where A_{tissue} is the activity of ^{177}Lu and ^{111}In in the tissue sample, corrected for radioactivity decay to the time of injection, and m_{tissue} is the mass of the tissue.

The mean tumor-to-normal-tissue activity concentration ratio, tumor/normal-tissue, at the time of injection was calculated:

$$Tumor/Normal-tissue = \frac{C_{tumour}}{C_{normal-tissue}}$$

To estimate the mean absorbed dose from ^{177}Lu-octreotate in the normal tissue and in the tumor the MIRD (Medical International Radiation Dose) (40) formalism was used:

$$\overline{D} = \frac{\widetilde{A}_{tissue} nE\phi}{m_{tissue}}$$

where $\tilde{A}_{tissue}$ is the cumulated activity i.e. the number of decays in the tissue source region during the time of interest, and was determined by estimating the area under curve for the injected activity concentration in each tissue vs. time. The product nE is the mean energy emitted per nuclear transformation (147 keV/decay for ^{177}Lu including the contribution from β particles, Auger and conversion electrons). ϕ is the absorbed fraction and was set to 1 (electrons), and *m* is the mass of the tissue sample.

Statistical analysis. All results are expressed as the mean value and standard error of the mean (SEM). Student's t-test was used to analyse data between groups. P-values <0.05 were considered statistically significant. Linear regression analysis was performed using IBM SPSS statistics 18 (IBM Co., NY, USA) for the relationship tumor uptake vs. tumor mass and tumor/blood vs. tumor mass.

Results

Biodistribution of ^{177}Lu-octreotate. The ^{177}Lu activity concentration in tissue samples from nude mice transplanted with GOT2 after injection of ^{177}Lu-octreotate is shown in Table I. The highest ^{177}Lu activity concentration was found in the kidneys. High activity concentration was also found in other tissues expressing SSTR, e.g. the tumor, adrenals and pancreas. The uptake in tumor tissue was lower than the in adrenals and pancreas, which also express SSTR. Blood, heart, liver, spleen and muscle had lower activity concentrations compared with the tissues mentioned at all time points studied. The activity concentration decreased with time after injection in the normal tissues as well as in the tumor. For most of the organs the activity concentration decreased faster between 24 h and 3 days, with a slower reduction rate between 3 day and 7 day. In adrenals, heart, pancreas and tumor the activity concentration decreased with the amount of activity (amount of peptide), with higher values for 5 MBq than for 10 MBq ($p<0.05$, except for adrenals at day 1), which in turn gave higher values than for 30 MBq at 24 h after injection ($p<0.05$).

The tumor/normal-tissue activity concentration ratio was highest for blood (12-26), and muscle (13-43) vs. all other tissues (Table II). The lowest tumor/normal-tissue value was

Table I. Activity concentration (%IA/g) of 5, 10 or 30 MBq ^{177}Lu-octreotate, corrected for radioactive decay, in different tissues in GOT2 tumor bearing nude mice at 24 h (n=5-6/group), 3 days (n=5-6/group) and 7 days (n=5-6/group) after injection.

	^{177}Lu activity concentration (%IA/g)						
	5 MBq			10 MBq			30 MBq
Time after injection	24 h	3 days	7 days	24 h	3 days	7 days	24 h
Tissue							
Adrenals	0.87 (0.17)	0.62 (0.08)	0.43 (0.05)	0.72 (0.10)	0.44 (0.03)	0.18 (0.02)	0.30 (0.01)
Blood	0.020 (0.006)	0.011 (0.004)	0.0027 (0.0007)	0.0099 (0.0023)	0.0049 (0.0038)	0.0016 (0.0002)	0.011 (0.002)
Heart	0.054 (0.004)	0.036 (0.004)	0.027 (0.002)	0.036 (0.001)	0.035 (0.002)	0.014 (0.001)	0.027 (0.003)
Kidneys	5.0 (0.1)	1.5 (0.3)	0.62 (0.13)	6.2 (0.3)	2.4 (0.3)	0.63 (0.11)	5.4 (0.5)
Liver	0.14 (0.01)	0.076 (0.006)	0.048 (0.002)	0.12 (0.004)	0.11 (0.01)	0.040 (0.003)	0.13 (0.01)
Muscle	0.012 (0.002)	0.057 (0.001)	0.0024 (0.0003)	0.023 (0.013)	0.0083 (0.0012)	0.0042 (0.0019)	0.01 (0.01)
Pancreas	2.0 (0.4)	0.70 (0.06)	0.28 (0.04)	0.77 (0.12)	0.48 (0.06)	0.095 (0.011)	0.48 (0.08)
Spleen	0.12 (0.01)	0.074 (0.009)	0.058 (0.006)	0.093 (0.006)	0.077 (0.007)	0.032 (0.002)	0.11 (0.02)
Tumor	0.37 (0.01)	0.21 (0.01)	0.094 (0.011)	0.23 (0.02)	0.086 (0.006)	0.039 (0.001)	0.13 (0.01)

Values are given as mean (SEM).

Table II. Tumor/normal-tissue activity concentration ratio (T/N) of 5, 10 or 30 MBq ^{177}Lu-octreotate, corrected for radioactive decay, in different tissues in nude mice bearing the GOT2 tumor at 24 h (n=5-6/group), 3 days (n=5-6/group) and 7 days (n=5-6/group) after injection.

	Tumor/normal-tissue ^{177}Lu activity concentration ratio (T/N)						
	5 MBq			10 MBq			30 MBq
Time after injection	24 h	3 days	7 days	24 h	3 days	7 days	24 h
Tissue							
Adrenals	0.51 (0.11)	0.35 (0.05)	0.24 (0.04)	0.36 (0.07)	0.27 (0.03)	0.20 (0.02)	0.43 (0.02)
Blood	23 (4)	29 (8)	46 (12)	26 (3)	27 (6)	48 (7)	12 (2)
Heart	7.0 (0.6)	6.1 (0.8)	3.5 (0.3)	6.6 (0.5)	3.8 (0.4)	2.8 (0.2)	4.8 (0.4)
Kidneys	0.073 (0.002)	0.18 (0.06)	0.18 (0.04)	0.038 (0.003)	0.040 (0.003)	0.048 (0.005)	0.023 (0.002)
Liver	2.8 (0.2)	2.8 (0.1)	2.0 (0.2)	2.0 (0.1)	1.3 (0.2)	0.93 (0.08)	0.97 (0.12)
Muscle	37 (7)	38 (4)	43 (11)	20 (4)	19 (4)	14 (4)	13 (1)
Pancreas	0.20 (0.03)	0.31 (0.03)	0.36 (0.06)	0.34 (0.06)	0.60 (0.12)	0.57 (0.09)	0.29 (0.05)
Spleen	3.2 (0.2)	3.0 (0.4)	1.7 (0.2)	2.6 (0.3)	1.4 (0.1)	0.97 (0.06)	1.2 (0.2)

Values are given as mean (SEM).

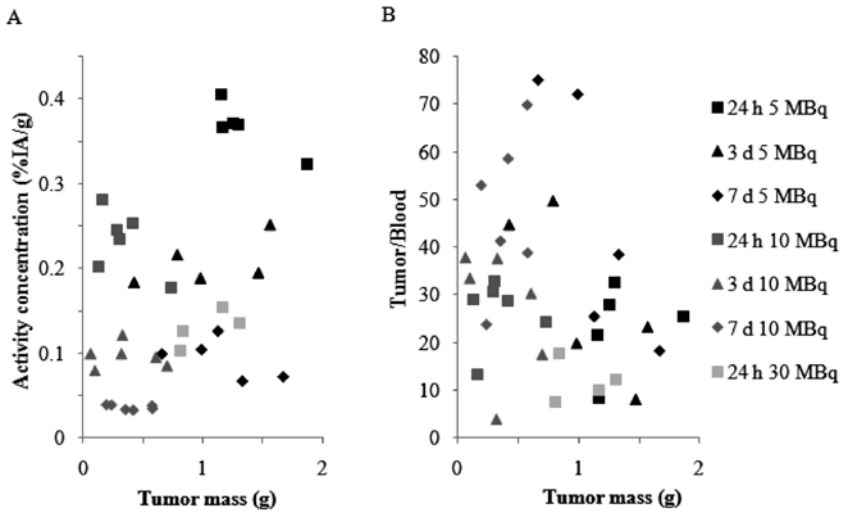

Figure 1. (A) Activity concentration (%IA/g) in GOT2 tumor tissue, and (B) corresponding tumor/blood ^{177}Lu activity concentration ratio at 24 h, 3 days, and 7 days after injection of 5, 10 or 30 MBq ^{177}Lu-octreotate in nude mice as a function of tumor mass (g). Values are corrected for radioactive decay. No relationship between tumor uptake vs. tumor mass and tumor/blood vs. tumor mass was found (p>0.05).

found for the kidneys. Initially (24 h after injection), tumor/normal-tissue was higher than unity for blood, heart, liver, muscle and spleen. The ratio increased with time for blood, kidneys, and pancreas (5 MBq), and remained constant, or decreased with time, for the other tissue types. The tumor/kidney and tumor/adrenals decreased with increased amount of activity/peptide at all points-in-time studied (p<0.05).

The linear regression analysis showed no correlation between tumor uptake and tumor mass for any peptide amount or time after injection, p>0.05 (Fig. 1A). Likewise, no relationship between tumor/blood ^{177}Lu activity concentration ratio and tumor mass was obtained, p>0.05 (Fig. 1B).

The mean absorbed dose from ^{177}Lu-octreotate in the tumor was 0.013 Gy/MBq. In comparison, the absorbed dose was higher in the adrenals (0.051 Gy/MBq), pancreas (0.036 Gy/MBq), and the kidneys (0.35 Gy/MBq) compared to the tumor.

Biodistribution of ^{111}In-MG0. The ^{111}In activity concentrations (C_{tissue}) and tumor/normal-tissue of ^{111}In-MG0 in nude mice transplanted with GOT2 are shown in Fig. 2A. The activity concentration was highest in kidneys and muscle followed by tumor. There was a significantly lower ^{111}In activity concentration in the tumor in the mice that also received an excess of unlabeled human minigastrin simultaneously with the ^{111}In-MG0 (0.32%IA/g vs. 0.79%IA/g, p=0.01). This effect could also be seen for adrenals, liver, lungs, and spleen (p<0.05).

After injection of ^{111}In-MG0 tumor/normal-tissue ratio was highest for heart and spleen, and values above unity were found for all tissue types except for the kidneys (Fig. 2B). In

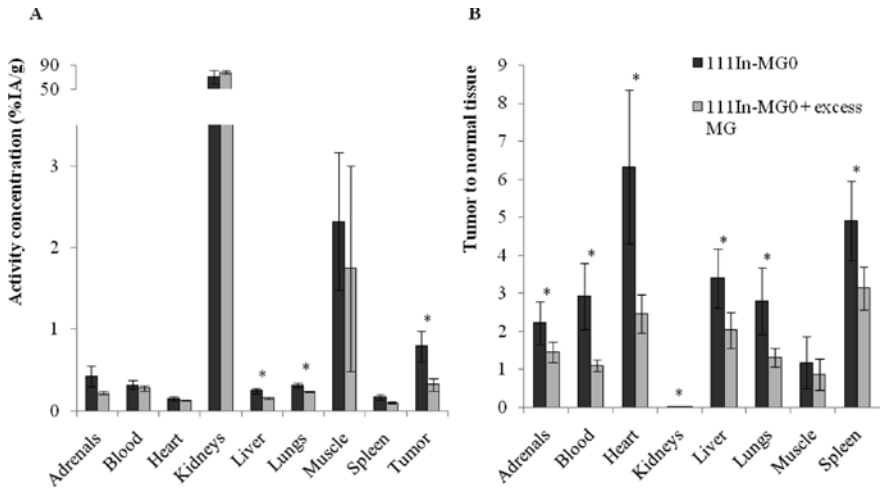

Figure 2. (A) ^{111}In activity concentration in GOT2 tumor tissue (%IA/g), corrected for radioactive decay, and (B) corresponding tumor/normal-tissue ^{111}In activity concentration ratios at 1 h after injection of ^{111}In-MG0 in GOT2 tumor bearing nude mice. *Statistically significant difference between the groups, $p<0.05$).

the mice that also received excess of unlabeled human minigastrin, significantly lower tumor/normal-tissue values were found for all tissue types except for muscle ($p<0.05$).

No correlation between activity concentration of ^{111}In-MG0 in tumor and tumor mass could be seen ($p>0.05$). This finding was also observed for tumor/blood vs. tumor mass ($p>0.05$).

Discussion

In this study we evaluated the biodistribution of radiolabeled octreotate (somatostatin analogue) and MG0 (gastrin analogue) in nude mice transplanted with GOT2 tumor. The origin of GOT2 was a tumor biopsy from a patient with MTC harvested at surgery. Many MTCs overexpress SSTR and CCK2/gastrin receptors (24-26,41). Somatostatin receptor scintigraphy can be used for localization of MTC prior to surgery (42), and therapy using radiolabeled somatostatin analogues have been suggested for patients with high SSTR expression and non-resectable tumor (41). Furthermore, many radiolabeled CCK2 and gastrin analogues are produced and tested for binding specificity in order to find new radiopharmaceuticals useful for localization of tumors expressing CCK2/gastrin receptors (12,31,32).

A moderate, but specific, uptake of ^{177}Lu-octreotate was demonstrated in the GOT2 tumors. No studies on ^{177}Lu-octreotate in other MTC animal models were found in the literature. When comparing the biodistribution data of ^{177}Lu-octreotate with corresponding data in animal models with other types of SSTR-expressing tumors differences between the models were found (Table III). The ^{177}Lu activity concentration in GOT2 was much lower than that of the midgut carcinoid GOT1 and small cell lung cancer (SCLC) H69 (about 45 and 10 times lower, respectively) based on studies performed in our laboratory (17,18). The reason is the very high SSTR expression in these cell lines and low SSTR expression in GOT2 (39). Also the activity concentrations in CA20948 in rats and AR42J in nude mice were higher than in GOT2 (43).

Saturation of the SSTR is an issue that must be considered when using radiolabeled somatostatin analogues in animal models. In the GOT1 mouse model with very high expression of SSTR2 and 5, saturation was found at peptide amounts higher than 1 μg octreotide (15). In the present study reduced ^{177}Lu activity concentration with increased amount of activity administered was found in GOT2 tumor tissue as well as in the SSTR expressing pancreas and adrenals. The effect was most prominent for pancreas with a reduction by a factor of 3 between the 5 MBq (0.2 μg) and 10 MBq (0.4 μg) groups. Since DOTA-octreotate has higher affinity for SSTR than DTPA-octreotide (44) and thus higher uptake in SSTR expressing tumors, the saturation should occur at even lower peptide amounts in the present study compared to GOT1 studies. In the present study it was not possible to define the level of possible saturation.

The high kidney uptake of ^{177}Lu-octreotate in the present study can be explained by the fact that kidneys in mice are one of few non-NE organs besides the pancreas that express all five SSTR (45-47). Further, there is a difference in the expression pattern of SSTR between human and mice kidneys, which complicates the translation of uptake data and dosimetry between species. Thus, high radionuclide concentration in kidneys in mice does not necessarily mean high uptake in human kidneys after injection of radiolabeled somatostatin analogues. On the other hand, the tumor/blood value in GOT2 model was higher than in AR42J model (23 vs. 13 at 1 day after injection); a similar relationship was found for tumor/kidney. Furthermore, tumor/blood and tumor/kidney increased with time for GOT2 and H69 (by a factor of 2-3), while it clearly decreased with time for CA20948 (by a factor of 3-5). This longer retention in GOT2 and H69 indicates a higher internalization in the tumor cells.

When comparing GOT2 with GOT1 model, only tumor/blood, tumor/kidney, tumor/muscle (5 MBq) and tumor/pancreas increased for GOT2 with time, while for GOT1 the tumor/normal-tissue values for all normal tissues studied increased with time 3-14 days after injection of ^{177}Lu-octreotide (14).

Table III. Biodistribution, given as ^{177}Lu activity concentration [%IA/g] in various tumor bearing animal models 1 and 7 days after injection of ^{177}Lu-octreotate.

Tumor type (animal)	GOT2 (nude mouse)		NCI-H69 (nude mouse)		GOT 1 (nude mouse)		AR42J (nude mouse)	CA20948 (rat)	
Study	Present study		Schmitt *et al* (18)		Kölby *et al* (17)		de Araújo *et al* (43)	Lewis *et al* (51)	
Injected activity (peptide amount)	5 MBq (0.2 μg)		3.3 MBq (0.7 μg)		7.5 MBq (0.25 μg)		0.74 MBq (1 μg)	1.3 MBq (0.67 μg)	
	^{177}Lu activity concentration (%IA/g)								
Time after injection	1 day	7 days	1 day	7 days	1 day	7 days	1 day	1 day	7 days
Tissue									
Adrenals	0.87	0.43	0.34	0.43	-	-	2.1[a]	0.21	0.11
Blood	0.020	0.0027	0.008	0.001	-	-	0.06	0.030	0.010
Heart	0.054	0.027	0.034	0.011	-	-	0.1	0.000	0.070
Kidneys	5.0	0.62	2.2	0.27	6-8	-	4.4	1.7	0.94
Liver	0.14	0.048	0.1	0.068	-	-	0.4	0.28	0.18
Muscle	0.012	0.0024	0.011	0.001	-	-	0.03	0.012	0.00
Pancreas	2.0	0.28	0.41	0.032	-	-	1.6	2.3	1.1
Spleen	0.12	0.058	0.12	0.032	-	-	0.3	0.010	0.010
Tumor	0.37	0.094	3.7	1.2	17	8	0.8	6.1	0.65

Data are corrected for physical decay. [a]In adrenals in this study the results were expressed as %IA/organ.

Table IV. Biodistribution, given as ^{111}In activity concentration [%IA/g] in various tumor bearing animal models (all nude mice) 1, 2 and 4 h after injection of ^{111}In-MG0 using either DOTA or DTPA as chelating agent.

Tumor type	GOT2	HEK293-CCK2i4svR	TT	A431-CCK2R	AR42J
Study	Present study	Lavermanet *al* (32)	Béhé *et al* (30)	Laverman *et al* (31)	Mather *et al* (50)
Chelate	DOTA	DOTA	DTPA	DOTA	DTPA
Injected activity (peptide amount)	22 kBq (20 ng)	370 kBq (20 ng)	555 kBq (10 μg)	370 kBq (0.03 nmol)	1300-1700 kBq (200 ng)
	^{111}In activity concentration (%IA/g)				
Time after injection	1 h	2 h	1 h	1 h	4 h
Tissue					
Adrenals	0.42	-	-	-	-
Blood	0.32	0.2-0.3	0.20	<0.1	0.06
Heart	0.15	-	-	-	-
Kidneys	71	60	54	55-60	60
Liver	0.24	1.2-1.3	0.12	-	-
Lungs	0.32	0.2-0.3	-	-	-
Muscle	2.3	<0.1	-	<0.1	-
Spleen	0.17	0.8-0.9	0.10	-	-
Tumor	0.79	6.1	0.45	13	2.6

Data are corrected for physical decay.

As a consequence of the lower GOT2 uptake the mean absorbed dose to the tumor from ^{177}Lu-octreotate was much lower for GOT2 tumors (0.011 Gy/MBq) than for GOT1 tumors (1.6-4.0 Gy/MBq) (17,48) and H69 tumors (0.29 Gy/MBq) (18). It should be noted that successful therapy has been achieved both in the GOT1 and H69 mouse models (17,19).

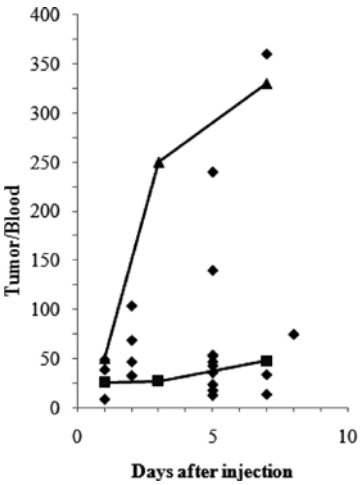

Figure 3. Tumor/blood ^{177}Lu activity concentration ratios obtained in nude mice transplanted with GOT2 (human MTC) after injection of ^{177}Lu-octreotate (■) compared to corresponding values for nude mice transplanted with GOT1 (human midgut carcinoid) after injection of ^{111}In-octreotide (▲) (14) and patients with MTC injected with ^{111}In-octreotide (♦) (41,49).

The tumor/blood values for GOT2 animal model was compared with corresponding values for patients with MTC tumors obtained from biopsies during surgery (41,49) in Fig. 3. The values in the model are clearly in accordance with the clinical data, although tumor/blood values for some of the patients were much higher than those obtained in GOT2 model. Therapy of MTC using ^{177}Lu-octreotate has been suggested for patients with high SSTR expression and non-resectable tumor (41). Based on the concordance of our clinical and GOT2 data, the xenograft model seems to be useful as a relevant test model, even though therapy would not be efficient in patients with such a low uptake as seen in GOT2 cells.

Regarding ^{111}In-MG0 there was a significantly lower uptake in GOT2 tumors in mice receiving an excess of unlabeled human minigastrin along with the ^{111}In-MG0, which indicates a specific binding to the CCK2/gastrin receptors. This finding was, however, also seen in most other organs. High kidney uptake and relatively low tumor uptake, as obtained in the GOT2 model with ^{111}In-MG0, have previously been shown for ^{111}In-DTPA-MG0 in nude mice bearing the human TT MTC cell line (71%IA/g and 0.79%IA/g, respectively, in the present study vs. 54%IA/g and 0.45%IA/g in the TT model after 1 h) (Table IV). The higher uptake and tumor/blood value in GOT2 vs. TT tumors might depend on higher receptor expression. Differences in affinity due to separate chelating agents used in the two studies cannot be excluded. Activity concentrations in other organs and tissues were similar but slightly lower in the TT model.

In comparison with other studies performed with ^{111}In-labeled MG0 on BALB/c mice, bearing various tumor types, the highest tumor uptake was found in the CCK2/gastrin receptor transfected cell lines (6.1 and 13%IA/g vs. 0.79%IA/g for GOT2 after 1 h) (31,32,50) (Table IV). The uptake in the rat endocrine pancreatic tumor model AR42J was higher than in GOT2, but lower than in the transfected tumors (50).

All studies with ^{111}In-labeled MG0 displayed high kidney uptake (55-70%IA/g after 1-4 h). One of the studies also demonstrated that co-injection of polyglutamic acids effectively reduced the kidney uptake of ^{111}In-DTPA-MG0 with up to 90% (30), which suggests that radiolabeled gastrin analogues might be useful for diagnosis, and possibly therapy, despite the high kidney uptake.

In the H69 model we previously found an inverse relationship at 24 h, but not at 3 and 7 days after injection of ^{177}Lu-octreotate between activity concentration and tumor mass, demonstrating a higher uptake in smaller tumors (18). No such correlations between activity concentration and tumor mass, or tumor/blood and tumor mass, were seen for GOT2 given ^{177}Lu-octreotate or ^{111}In-MG0.

Despite the relatively moderate uptake of both ^{177}Lu-octreotate and ^{111}In-MG0 in GOT2 tumors in mice, the conclusion is that this xenograft model may be suitable for the evaluation of radiolabeled somatostatin, CCK and gastrin analogues for diagnosis and therapy of MTC. The possibility of blocking the uptake of radionuclide in the kidney should be considered.

Acknowledgements

The authors thank Lilian Karlsson and Ann Wikström for their skilled performance of the animal studies. We also thank Dr Jonas Nilsson for valuable help with the radiolabeling of MG0. This study was supported by grants from the European Commission FP7 Collaborative Project TARCC HEALTH-F2-2007-201962, Swedish Research Council, the Swedish Cancer Society, and BioCARE - a National Strategic Research Program at University of Gothenburg, the King Gustav V Jubilee Clinic Cancer Research Foundation, and the Assar Gabrielsson Research Foundation. The study was performed within the EC COST Action BM0607.

References

1. Kwekkeboom DJ, Kam BL, van Essen M, *et al*: Somatostatin-receptor-based imaging and therapy of gastroenteropancreatic neuroendocrine tumors. Endocr Relat Cancer 17: R53-P73, 2010.
2. Kolby L, Bernhardt P, Ahlman H, *et al*: A transplantable human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. Am J Pathol 158: 745-755, 2001.
3. Nilsson O, Kolby L, Bernhardt P, Forssell-Aronsson E, Johanson V and Ahlman H: GOT1 xenografted to nude mice: a unique model for in vivo studies on SSTR-mediated radiation therapy of carcinoid tumors. Ann N Y Acad Sci 1014: 275-279, 2004.
4. Taylor JE, Theveniau MA, Bashirzadeh R, Reisine T and Eden PA: Detection of somatostatin receptor subtype 2 (SSTR2) in established tumors and tumor cell lines: evidence for SSTR2 heterogeneity. Peptides 15: 1229-1236, 1994.
5. Hashemi SH, Benjegard SA, Ahlman H, *et al*: 111In-labelled octreotide binding by the somatostatin receptor subtype 2 in neuroendocrine tumours. Br J Surg 90: 549-554, 2003.
6. Evers BM, Hurlbut SC, Tyring SK, Townsend CM Jr, Uchida T and Thompson JC: Novel therapy for the treatment of human carcinoid. Ann Surg 213: 411-416, 1991.
7. Evers BM, Townsend CM Jr, Upp JR, *et al*: Establishment and characterization of a human carcinoid in nude mice and effect of various agents on tumor growth. Gastroenterology 101: 303-311, 1991.
8. Pfragner R, Wirnsberger G, Niederle B, *et al*: Establishment of a continuous cell line from a human carcinoid of the small intestine (KRJ-I). Int J Oncol 8: 513-520, 1996.
9. Modlin IM, Kidd M, Pfragner R, Eick GN and Champaneria MC: The functional characterization of normal and neoplastic human enterochromaffin cells. J Clin Endocrinol Metab 91: 2340-2348, 2006.

10. Leong SS, Horoszewicz HJ, Friedman M, *et al*: A new cell-line for studies on human thyroid medullary carcinoma. Proc Am Assoc Cancer Res 22: 49-49, 1981.
11. Olszewska-Pazdrak B, Townsend CM Jr and Hellmich MR: Agonist-independent activation of Src tyrosine kinase by a cholecystokinin-2 (CCK2) receptor splice variant. J Biol Chem 279: 40400-40404, 2004.
12. Aloj L, Caraco C, Panico M, *et al*: In vitro and in vivo evaluation of 111In-DTPAGlu-G-CCK8 for cholecystokinin-B receptor imaging. J Nucl Med: 45: 485-494, 2004.
13. Aloj L, Aurilio M, Rinaldi V, *et al*: Comparison of the binding and internalization properties of 12 DOTA-coupled and (111) In-labelled CCK2/gastrin receptor binding peptides: a collaborative project under COST Action BM0607. Eur J Nucl Med Mol Imaging 38: 1417-1425, 2011.
14. Bernhardt P, Kolby L, Johanson V, *et al*: Biokinetics of 111In-DTPA-D-Phe(1)-octreotide in nude mice transplanted with a human carcinoid tumor. Nucl Med Biol 28: 67-73, 2001.
15. Bernhardt P, Kolby L, Johanson V, Nilsson O, Ahlman H and Forssell-Aronsson E: Biodistribution of 111in-DTPA-D-Phe1-octreotide in tumor-bearing nude mice: influence of amount injected and route of administration. Nucl Med Biol 30: 253-260, 2003.
16. Bernhardt P, Oddstig J, Kolby L, Nilsson O, Ahlman H and Forssell-Aronsson E: Effects of treatment with (177) Lu-DOTA-Tyr(3)-octreotate on uptake of subsequent injection in carcinoid-bearing nude mice. Cancer Biother Radiopharm 22: 644-653, 2007.
17. Kolby L, Bernhardt P, Johanson V, *et al*: Successful receptor-mediated radiation therapy of xenografted human midgut carcinoid tumour. Br J Cancer 93: 1144-1151, 2005.
18. Schmitt A, Bernhardt P, Nilsson O, *et al*: Biodistribution and dosimetry of 177Lu-labeled [DOTA0,Tyr3]octreotate in male nude mice with human small cell lung cancer. Cancer Biother Radiopharm 18: 593-599, 2003.
19. Schmitt A, Bernhardt P, Nilsson O, *et al*: Radiation therapy of small cell lung cancer with 177Lu-DOTA-Tyr3-octreotate in an animal model. J Nucl Med 45: 1542-1548, 2004.
20. Schmitt A: Experimental studies of 177Lu-DOTA0,Tyr3-octreotate for radionuclide therapy of small cell lung cancer. Licentiate Thesis Department of Radiation Physics, Göteborg University, Göteborg. 2005.
21. Schmitt A, Bernhardt P, Nilsson O, Ahlman H, Kolby L and Forssell-Aronsson E: Differences in biodistribution between 99mTc-depreotide, 111In-DTPA-octreotide, and 177Lu-DOTA-Tyr3-octreotate in a small cell lung cancer animal model. Cancer Biother Radiopharm 20: 231-236, 2005.
22. Taal BG, Hoefnagel C, Boot H, Valdes Olmos R and Rutgers M: Improved effect of 131I-MIBG treatment by predosing with non-radiolabeled MIBG in carcinoid patients, and studies in xenografted mice. Ann Oncol 11: 1437-1443, 2000.
23. Kolby L, Bernhardt P, Levin-Jakobsen AM, *et al*: Uptake of meta-iodobenzylguanidine in neuroendocrine tumours is mediated by vesicular monoamine transporters. Br J Cancer 89: 1383-1388, 2003.
24. Amiri-Mosavi A, Ahlman H, Tisell LE, *et al*: Expression of cholecystokinin-B/gastrin receptors in medullary thyroid cancer. Eur J Surg 165: 628-631, 1999.
25. Reubi JC, Schaer JC and Waser B: Cholecystokinin(CCK)-A and CCK-B/gastrin receptors in human tumors. Cancer Res 57: 1377-1386, 1997.
26. Reubi JC and Waser B: Unexpected high incidence of cholecystokinin-B/gastrin receptors in human medullary thyroid carcinomas. Int J Cancer 67: 644-647, 1996.
27. Behe M and Behr TM: Cholecystokinin-B (CCK-B)/gastrin receptor targeting peptides for staging and therapy of medullary thyroid cancer and other CCK-B receptor expressing malignancies. Biopolymers 66: 399-418, 2002.
28. Behr TM, Jenner N, Radetzky S, *et al*: Targeting of cholecystokinin-B/gastrin receptors in vivo: preclinical and initial clinical evaluation of the diagnostic and therapeutic potential of radiolabelled gastrin. Eur J Nucl Med 25: 424-430, 1998.
29. Behr TM, Jenner N, Behe M, *et al*: Radiolabeled peptides for targeting cholecystokinin-B/gastrin receptor-expressing tumors. J Nucl Med 40: 1029-1044, 1999.
30. Behe M, Kluge G, Becker W, Gotthardt M and Behr TM: Use of polyglutamic acids to reduce uptake of radiometal-labeled minigastrin in the kidneys. J Nucl Med 46: 1012-1015, 2005.
31. Laverman P, Joosten L, Eek A, *et al*: Comparative biodistribution of 12 (111)In-labelled gastrin/CCK2 receptor-targeting peptides. Eur J Nucl Med Mol Imaging 38: 1410-1416, 2011.
32. Laverman P, Roosenburg S, Gotthardt M, *et al*: Targeting of a CCK(2) receptor splice variant with (111)In-labelled cholecystokinin-8 (CCK8) and (111)In-labelled minigastrin. Eur J Nucl Med Mol Imaging 35: 386-392, 2008.
33. Froberg AC, de Jong M, Nock BA, *et al*: Comparison of three radiolabelled peptide analogues for CCK-2 receptor scintigraphy in medullary thyroid carcinoma. Eur J Nucl Med Mol Imaging 36: 1265-1272, 2009.
34. Nock BA, Maina T, Behe M, *et al*: CCK-2/gastrin receptor-targeted tumor imaging with (99m)Tc-labeled minigastrin analogs. J Nucl Med 46: 1727-1736, 2005.
35. Gotthardt M, Behe MP, Beuter D, *et al*: Improved tumour detection by gastrin receptor scintigraphy in patients with metastasised medullary thyroid carcinoma. Eur J Nucl Med Mol Imaging 33: 1273-1279, 2006.
36. Logsdon CD, Moessner J, Williams JA and Goldfine ID: Glucocorticoids increase amylase mRNA levels, secretory organelles, and secretion in pancreatic acinar AR42J cells. J Cell Biol 100: 1200-1208, 1985.
37. Longnecker DS, Lilja HS, French J, Kuhlmann E and Noll W: Transplantation of azaserine-induced carcinomas of pancreas in rats. Cancer Lett 7: 197-202, 1979.
38. Roebuck BD, Yager JD Jr and Longnecker DS: Dietary modulation of azaserine-induced pancreatic carcinogenesis in the rat. Cancer Res 41: 888-893, 1981.
39. Johanson V, Ahlman H, Bernhardt P, *et al*: A transplantable human medullary thyroid carcinoma as a model for RET tyrosine kinase-driven tumorigenesis. Endocr Relat Cancer 14: 433-444, 2007.
40. Bolch WE, Eckerman KF, Sgouros G and Thomas SR: MIRD pamphlet No. 21: a generalized schema for radiopharmaceutical dosimetry-standardization of nomenclature. J Nucl Med 50: 477-484, 2009.
41. Forssell-Aronsson EB, Nilsson O, Bejegard SA, *et al*: 111In-DTPA-D-Phe1-octreotide binding and somatostatin receptor subtypes in thyroid tumors. J Nucl Med 41: 636-642, 2000.
42. Tisell LE, Ahlman H, Wangberg B, *et al*: Somatostatin receptor scintigraphy in medullary thyroid carcinoma. Br J Surg 84: 543-547, 1997.
43. de Araujo EB, Caldeira Filho JS, Nagamati LT, *et al*: A comparative study of 131I and 177Lu labeled somatostatin analogues for therapy of neuroendocrine tumours. Appl Radiat Isot: 67: 227-233, 2009.
44. Reubi JC, Schar JC, Waser B, *et al*: Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. Eur J Nucl Med 27: 273-282, 2000.
45. Bates CM, Kegg H and Grady S: Expression of somatostatin in the adult and developing mouse kidney. Kidney Int 66: 1785-1793, 2004.
46. Bates CM, Kegg H and Grady S: Expression of somatostatin receptors 1 and 2 in the adult mouse kidney. Regul Pept 119: 11-20, 2004.
47. Bates CM, Kegg H, Petrevski C and Grady S: Expression of somatostatin receptors 3, 4, and 5 in mouse kidney proximal tubules. Kidney Int 63: 53-63, 2003.
48. Sward C, Bernhardt P, Johanson V, *et al*: Comparison of [177Lu-DOTA0,Tyr3]-octreotate and [177Lu-DOTA0,Tyr3]-octreotide for receptor-mediated radiation therapy of the xenografted human midgut carcinoid tumor GOT1. Cancer Biother Radiopharm 23: 114-120, 2008.
49. Forssell-Aronsson E, Bernhardt P, Nilsson O, Tisell LE, Wangberg B and Ahlman H: Biodistribution data from 100 patients i.v. injected with 111In-DTPA-D-Phe1-octreotide. Acta Oncol 43: 436-442, 2004.
50. Mather SJ, McKenzie AJ, Sosabowski JK, Morris TM, Ellison D and Watson SA: Selection of radiolabeled gastrin analogs for peptide receptor-targeted radionuclide therapy. J Nucl Med 48: 615-622, 2007.
51. Lewis JS, Wang M, Laforest R, *et al*: Toxicity and dosimetry of (177)Lu-DOTA-Y3-octreotate in a rat model. Int J Cancer 94: 873-877, 2001.