Nephrotoxicity in Mice After Repeated Imaging Using ^{111}In-Labeled Peptides

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We determined the renal radiation dose of a series of ^{111}In-labeled peptides using animal SPECT. Because the animals' health deteriorated, renal toxicity was assessed. **Methods:** Wild-type and megalin-deficient mice were imaged repeatedly at 3- to 6-wk intervals to quantify renal retention after injection of 40–50 MBq of ^{111}In-diethylenetriaminepentaacetic acid–labeled peptides (octreotide, exendin, octreotate, neurotensin, and minigastrin analogs), and the absorbed kidney radiation doses were estimated. Body weight, renal function parameters, and renal histology were determined at 16–20 wk after the first scan and compared with those in naive animals. **Results:** Because of high renal retention, ^{111}In-diethylenetriaminepentaacetic acid–exendin-4 scans resulted in a 70-Gy kidney radiation dose in wild-type mice. Megalin-deficient kidneys received 20–40 Gy. The other peptides resulted in much lower renal doses. Kidney function monitoring indicated renal damage in imaged animals. **Conclusion:** Micro-SPECT enables longitudinal studies in 1 animal. However, long-term nephrotoxic effects may be induced after high renal radiation doses, even with ^{111}In-labeled radiotracers.

Key Words: nephrotoxicity; ^{111}In; renal reabsorption; radiolabeled peptide; dosimetry

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Malignant cells often express a high density of membrane receptors for regulatory peptides such as somatostatin, gastrin, and exendin (*1*). Radiolabeled peptide analogs specifically targeting these receptors are used as either diagnostic or therapeutic tools in tumor imaging and radionuclide therapy. Peptide receptor radionuclide therapy (PRRT) using ^{90}Y- or ^{177}Lu-labeled somatostatin analogs (Tyr^3-octreotide or Tyr^3-octreotate) have led to significant therapeutic results in terms of tumor response, survival, and quality of life (*2*). ^{90}Y and ^{177}Lu both are β-particle–emitting radionuclides, whereas ^{111}In emits mainly γ-rays and additionally Auger and conversion electrons (CEs). The mean range in tissue is 3,900 μm (β^-) and 200 μm (β^-) for ^{90}Y and ^{177}Lu, respectively, and 0.02–10 μm (Auger) and 200–500 μm (CEs) for ^{111}In. When ^{111}In-labeled analogs were applied during PRRT, less therapeutic benefit was generated (*3,4*), probably because of the short range of the high linear energy transfer of Auger electrons, without cross-fire effect. In line with these observations, ^{111}In-octreotate was more efficacious for the treatment of smaller, than larger, tumors in CA20948 tumor–bearing rats (*5*).

A potential side effect of PRRT with somatostatin analogs is late renal damage due to partial renal retention of radioactivity in the proximal tubules. Nephrotoxic effects in patients have been described after administration of ^{90}Y-PRRT (*6*), but rarely after ^{177}Lu-PRRT (*7*), and not after ^{111}In-PRRT (*3*).

The scavenging receptor megalin, facilitating the renal reabsorption of many proteins and peptides in the proximal tubules, has proven to be essential for renal reabsorption of radiolabeled octreotide in mice. The uptake of ^{111}In-octreotide was significantly less in kidney-specific megalin-deficient mice than in wild-type (WT) mice in biodistribution studies (*8*), similar to the reducing effect of the coadministration of lysine in WT animals (*9*). In this study, we examined the role of megalin in the renal retention of ^{111}In-labeled somatostatin analogs and other peptides by imaging WT and megalin-deficient mice using a dedicated small-animal micro-SPECT/CT camera. In consecutive experiments, 5 different ^{111}In-labeled peptide analogs were administered to the same animals at intervals of at least 3 wk (*10*). Relatively high-activity doses of ^{111}In (40–50 MBq per mouse) were injected, permitting the accurate quantification of renal radioactivity. The renal uptake of all tested peptides in megalin-deficient mice was only 23%−62% of the uptake in WT mice (*10*).

The purpose of the current study was to assess the effects of high-dose SPECT using ^{111}In-labeled peptide analogs with regard to risk of nephrotoxicity. The total absorbed radiation dose to the kidneys of WT and megalin-deficient

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TABLE 1. Structure of Investigated Peptide Analogs, Their Molecular Weight, and Manufacturers

Peptide	Structure	Molecular weight	Company
Octreotide	DTPA-Tyr^3-octreotide	1,000	Covidien; Petten, The Netherlands
Octreotate	DTPA-Tyr^3-octreotate	1,000	Mallinckrodt; St. Louis, MO
Exendin	DTPA-Lys^{40}-exendin-4	4,200	Peptide Specialty Laboratories GmbH; Heidelberg, Germany
Neurotensin	DTPA-G(Pip)^6,G(Pam)^8,tBuG^{12}-neurotensin$^{6–13}$	815	BioSynthema, St. Louis, MO
Minigastrin 0	DTPA-D-Glu-(Glu)$_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH$_2$)	1,832	Kindly provided by Dr. Martin Behe; Marburg, Germany

mice was calculated. These results were correlated with renal histology and kidney function parameters, which were determined approximately 20 wk after the start of the experiment.

MATERIALS AND METHODS

Animals

Animal studies were conducted in accordance with the guidelines of the Animal Welfare Committee of both medical centers. Kidney-specific megalin-deficient mice (Megalin$^{lox/lox}$, apoE^{Cre}), generated as described previously (*11*), were kindly provided by Thomas E. Willnow (Berlin, Germany). Animals were bred locally, and animals expressing the apoE^{Cre} gene were identified by polymerase chain reaction analysis. Megalin-deficient animals were compared with WT C57BL/6 mice (Harlan); 5 animals of each strain and sex were imaged. Urine, serum, and kidneys from naive animals were tested for reference values.

Radionuclides and Peptides

$^{111}InCl_3$ was purchased from Covidien. Diethylenetriamine-pentaacetic acid (DTPA) peptides, as listed in Table 1, were radiolabeled as described previously (*12,13*). Specific activity was 20 MBq/nmol of peptide, with a radiochemical yield exceeding 95%, as confirmed by thin-layer chromatography (*12*).

Molecular Imaging

Mice were imaged using a 4-head multipinhole SPECT/CT camera (NanoSPECT/CT; Bioscan Inc.). Scans were obtained at 3 and 24 h after the injection of 40–50 MBq of ^{111}In-DTPA-peptide (2 nmol) while the animals were anesthetized with isoflurane/O_2 and body temperature was maintained using a heated bed. The imaging procedure was repeated in these animals after at least 3 wk of decay of ^{111}In. The order and intervals of the experiments are listed in Table 2. After the final scan, the animals were euthanized; relevant tissues were dissected and weighed, and their activity was determined in a γ-counter.

Nine-pinhole apertures with a diameter of 1.4 mm were used on each head of the camera, and the field of view was 16 mm. On the basis of the CT topogram, a body range of 30 mm covering the renal region was scanned in 24 min, with 120 s per projection. Settings of the ^{111}In energy peaks were 171 and 245 keV. The amount of radioactivity in a volume of interest of the kidneys was quantified using InVivoScope software (Bioscan, Inc.). Detected counts were converted to megabecquerels using a correction factor obtained by scanning a phantom, filled with a known amount of ^{111}In activity, with the same volume as the mouse body to correct for attenuation.

Dosimetry

The mathematic dosimetry model for mice as developed by Hindorf et al. (*14*) was used. The residence time of ^{111}In was assumed to follow a single exponential clearance pattern. Furthermore, only the kidney self-radiation dose was assumed. The renal uptake of ^{111}In-labeled octreotide in male mice and ^{111}In-labeled neurotensin in both sexes was too low to quantify at 24 h after injection; therefore, the clearance rate was assumed to be similar to that of ^{111}In-labeled octreotide in female mice.

Analytic Procedures

Urinary protein was measured using a commercially available colorimetric assay purchased from BioRad. Urine was collected for 24 h in metabolic cages, 12 wk (male mice) or 16 wk (female mice) after ^{111}In-DTPA-exendin-4 imaging. At euthanasia, 4 or 3 wk later, respectively, serum was collected to determine urea

TABLE 2. Absorbed Kidney Radiation Doses (Mean ± SD) After Subsequent Injection of ^{111}In-Labeled Peptides

	Female mice			Male mice		
		Absorbed kidney dose (Gy)			Absorbed kidney dose (Gy)	
Peptide	Time (wk)	WT	Megalin-deficient	Time (wk)	WT	Megalin-deficient
Octreotide	0	2.1 ± 0.5	1.0 ± 0.2	0	1.9 ± 0.5*	0.4 ± 0.2*
Exendin	4	73 ± 9	38 ± 11	4	72 ± 6	21 ± 4
Octreotate	13	3.3 ± 1.0	1.1 ± 0.3	9	2.9 ± 0.6	0.5 ± 0.1
Neurotensin	20	0.8 ± 0.2*	0.16 ± 0.04*	13	0.7 ± 0.2*	0.16 ± 0.04*
Minigastrin	23	28 ± 4	5 ± 3	20	30 ± 2	7 ± 1

*Clearance assumed to be equivalent to octreotide in females.
Data are mean ± SD.

TABLE 3. Calculation of Absorbed Kidney Dose After ^{111}In-DTPA-Exendin-4 Administration

Mouse group	Injected activity (MBq)	3 h after injection: Total MBq	3 h after injection: % Injected activity	24 h after injection: Total MBq	24 h after injection: % Injected activity	Kidney mass (mg)	mGy/MBq	Absorbed dose (Gy)
Female								
WT	42.4 ± 3.0	27.2 ± 2.6	64.0 ± 2.2	15.5 ± 1.7	44.4 ± 2.9	140 ± 13	1.66 ± 0.15	73 ± 9
Megalin-deficient	42.6 ± 4.7	16.1 ± 3.9	37.8 ± 8.1	8.5 ± 2.4	24.6 ± 6.2	156 ± 17	0.86 ± 0.23	38 ± 11
Male								
WT	39.2 ± 3.5	33.2 ± 3.3	69.3 ± 1.4	16.3 ± 1.4	42.7 ± 3.5	189 ± 11	1.46 ± 0.10	72 ± 6
Megalin-deficient	35.5 ± 3.1	17.5 ± 2.9	42.0 ± 8.2	7.0 ± 1.3	20.7 ± 4.8	225 ± 14	0.50 ± 0.11	21 ± 4

Data are mean ± SD.

and creatinine levels using standard clinical chemistry procedures.

Histology

One of the dissected kidneys was fixed in 10% buffered formalin and embedded in paraffin. Sections (4 μm) were cut, stained with hematoxylin-eosin or periodic acid-Schiff reagent, and evaluated microscopically to score renal damage according to a scale from grade 0 (no damage) to grade 4 (severe damage), as described previously (*15*).

RESULTS

The absolute amount of retained radioactivity in WT female controls 3 h after injection ranged from 5% (neurotensin) to 10%−15% (somatostatin analogs), 65% (minigastrin), and 225% (exendin) injected activity per gram of kidney. All peptides tested showed less renal uptake in megalin-deficient mice than in WT controls (reduction ranging from ~40% [exendin] to ~55% [minigastrin], ~60% [somatostatin], and ~75% [neurotensin]) (*10*). The uptake values combined with the S value (*14*), related to kidney mass as determined after euthanasia, were used to calculate the absorbed kidney dose after each scan. As an example, this calculation is shown in Table 3 for ^{111}In-DTPA-exendin-4, resulting in absorbed kidney radiation doses ranging from 20–40 Gy in megalin-deficient to more than 70 Gy in WT mice (Table 2).

Surveillance of the animals revealed that female WT mice showed some subdued but still responsive behavior approximately 16 wk after this dose. Body weight loss was observed as well (data not shown). The relationship with increased urinary protein levels was not significant, but a trend of elevated levels in imaged animals was found (Fig. 1). Some weeks later, serum urea and creatinine levels were found to be elevated in scanned animals—creatinine both in WT and in megalin-deficient mice, urea only in WT animals (Fig. 1). Grading of histologic renal damage revealed that the most pronounced effects were found in scanned female WT mice, showing thickening and necrosis of tubular basal lamina and glomerulosclerosis. In megalin-deficient mice, similar histologic damage was detected but less frequently (Fig. 2).

DISCUSSION

Recent improvements in resolution and sensitivity of small-animal SPECT cameras opened new possibilities in preclinical research, including serial imaging of the same

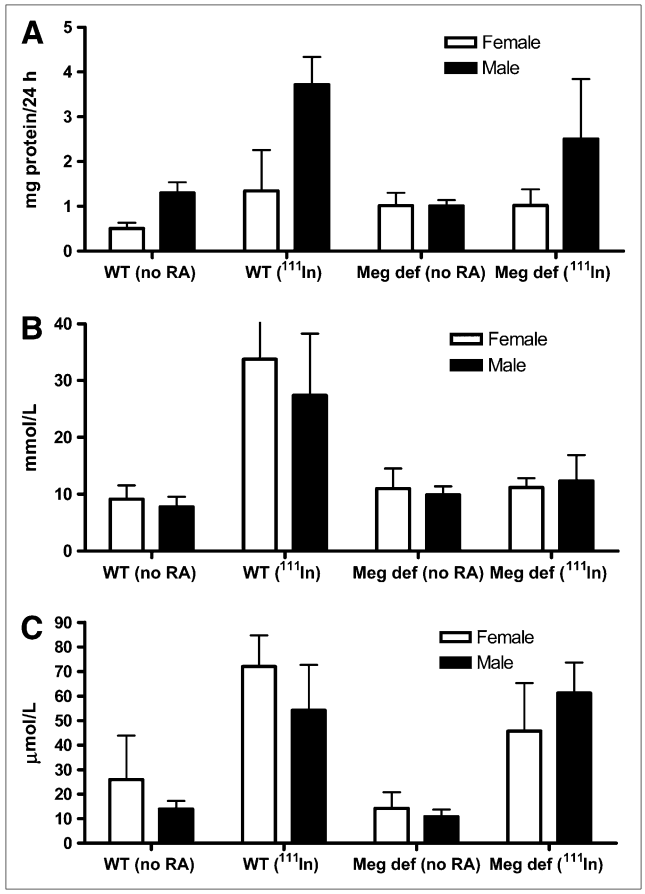

FIGURE 1. Urine and serum chemistry of both sexes of WT and kidney-specific megalin-deficient mice, either non-scanned (no RA) or scanned (^{111}In). (A) Urinary protein (mg/24 h) measured 12–16 wk after ^{111}In-DTPA-exendin-4 imaging (B and C). Urea in serum (mmol/L) (B) and creatinine in serum (μmol/L) (C), both determined at 16–19 wk after ^{111}In-DTPA-exendin-4 imaging. n = 2–8 per group. Values are presented as mean ± SD. Meg def = megalin-deficient.

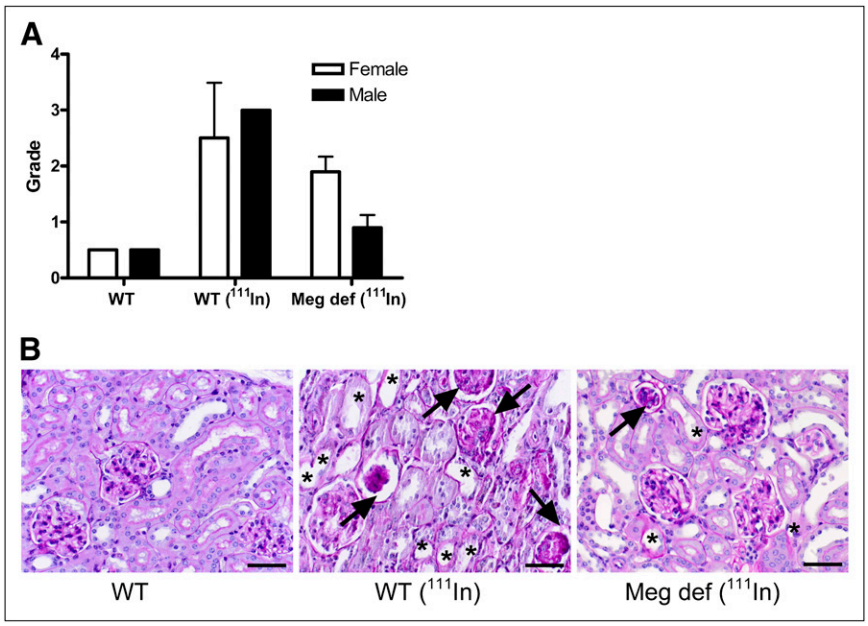

FIGURE 2. (A) Renal damage score of both sexes of WT and kidney-specific megalin-deficient mice, either non-scanned (no RA) or scanned (^{111}In). Kidneys of scanned mice were removed at 16–19 wk after ^{111}In-DTPA-exendin-4 imaging. Scores are based on histologic examination of periodic acid-Schiff–stained sections according to grading scale from 0 to 4 (*15*). (B) Examples of periodic acid-Schiff–stained, 4-μm paraffin-embedded kidney sections (×250, bar = 50 μm). Arrow points to glomerulosclerosis. n = 2–5 per group. Values are presented as mean ± SD. Meg def = megalin-deficient; * = tubular basal lamina thickening or necrosis of tubular epithelial cells.

animal, leading to reduction of animal numbers (*16*). In this study in mice, we quantified renal uptake of several ^{111}In-labeled peptides, enabling dosimetric calculations. Although the sensitivity of multipinhole micro-SPECT cameras is relatively high, we aimed for 1 MBq in the targeted organ to allow accurate quantification. In this comparative study, we administered 40–50 MBq of each ^{111}In-labeled peptide to achieve reliable quantification of renal retention, even in megalin-deficient mice with an expected renal uptake lower than that in WT mice. However, renal damage developed at approximately 16 wk after an unexpectedly high (38–70 Gy) kidney radiation dose. On the basis of these results, we had to repeat imaging of some peptides in naive animals to exclude the possibility that results were affected by impaired renal function.

Patients treated with ^{111}In-DTPA-octreotide PRRT did not encounter long-term nephrotoxic effects after a cumulative kidney dose of 45 Gy (*3*), whereas a safe kidney dose limit of 23 Gy was adapted from external-beam radiation (*17*). These patients acquired the total ^{111}In dose, fractionated in a minimum of 8 cycles with at least 2-wk intervals, whereas the mice in the current study received the 40- to 70-Gy kidney dose after a single administration. Kidney protection by dose fractionation also was observed after ^{177}Lu-octreotate PRRT in rats (*15*).

Dose calculations were based on published S values for ^{111}In in mouse kidneys (*14*); however, other authors described different, but comparable, S values (*18,19*). All are voxel-based models using either geometric shapes representing organs (*14*) or more realistic shapes (*18,19*), assuming homogeneous distribution of the radioactivity. This may lead to the underestimation of the effective dose, because renal radioactivity is primarily localized in the cortex, resulting in higher radiation doses to the tubuli (and glomeruli) (*20*). Another reason for dose underestimation might be the assumption of exponential clearance based on only 2 time points. Acquiring images at more, especially later, time points will provide better insight into the clearance and residence time of radiolabeled peptides and lead to more accurate dosimetry. In addition, biodistribution studies should be performed when relatively low renal uptake has to be quantified (*12*). In humans, the total kidney dose delivered by ^{111}In is partially due to γ-irradiation; this dose is less important in mice because of much smaller kidneys. Taken together, these issues hamper comparison between mice and humans concerning the effects of absorbed kidney doses.

Body weight loss was the first indication of nephrotoxicity in these mice. Renal dysfunction was confirmed by elevated urinary protein content and by elevated urea and creatinine serum levels, both to a moderate extent. Microscopic evaluation of kidney sections revealed histologic damage in both tubuli and glomeruli. So, after megalin-mediated reabsorption of ^{111}In-labeled peptides into proximal tubular cells, the glomeruli were also affected. The maximal range of Auger electrons (0.02–10 μm), and especially the longer range of CEs (200–500 μm), is sufficient to reach adjacent glomeruli. In the future—in addition to the chemical and histologic studies that have been performed (*16*)—we will perform renographic studies using ^{99m}Tc-dimercaptosuccinic acid, ^{99m}Tc-mercaptoacetyltriglycine, and ^{111}In-DTPA to gain insight into both tubular and glomerular function after high absorbed kidney doses.

In retrospect ^{111}In-DTPA-exendin-4 imaging should have been performed as the last peptide in the series, and the injected activity dose could have been less—10 MBq instead of 40–50 MBq.

CONCLUSION

An absorbed kidney dose of more than 40 Gy due to renal accumulation of ^{111}In-labeled peptides resulted in long-term nephrotoxicity in mice. The small-range, low-energy Auger electrons and CEs emitted by ^{111}In caused both tubular and glomerular damage. Micro-SPECT offers the opportunity to perform follow-up studies in 1 animal by consecutive scanning. However, the risk of side effects due to high radiation doses must be considered.

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REFERENCES

1. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev.* 2003;24:389–427.
2. van Essen M, Krenning EP, Kam BL, et al. Peptide-receptor radionuclide therapy for endocrine tumors. *Nat Rev Endocrinol.* 2009;5:382–393.
3. Valkema R, De Jong M, Bakker WH, et al. Phase I study of peptide receptor radionuclide therapy with [In-DTPA]octreotide: the Rotterdam experience. *Semin Nucl Med.* 2002;32:110–122.
4. Anthony LB, Woltering EA, Espenan GD, et al. Indium-111-pentetreotide prolongs survival in gastroenteropancreatic malignancies. *Semin Nucl Med.* 2002;32:123–132.
5. Capello A, Krenning E, Bernard B, et al. ^{111}In-labelled somatostatin analogues in a rat tumour model: somatostatin receptor status and effects of peptide receptor radionuclide therapy. *Eur J Nucl Med Mol Imaging.* 2005;32:1288–1295.
6. Otte A, Herrmann R, Heppeler A, et al. Yttrium-90 DOTATOC: first clinical results. *Eur J Nucl Med.* 1999;26:1439–1447.
7. Kwekkeboom DJ, de Herder WW, Kam BL, et al. Treatment with the radiolabeled somatostatin analog [177 Lu-DOTA 0,Tyr3]octreotate: toxicity, efficacy, and survival. *J Clin Oncol.* 2008;26:2124–2130.
8. de Jong M, Barone R, Krenning E, et al. Megalin is essential for renal proximal tubule reabsorption of ^{111}In-DTPA-octreotide. *J Nucl Med.* 2005;46:1696–1700.
9. Melis M, Krenning EP, Bernard BF, et al. Localisation and mechanism of renal retention of radiolabelled somatostatin analogues. *Eur J Nucl Med Mol Imaging.* 2005;32:1136–1143.
10. Vegt E, Melis M, Eek A, et al. Megalin plays an important role in the renal uptake of various radiolabelled peptides: studies in megalin-deficient mice [abstract]. *Eur J Nucl Med Mol Imaging.* 2009;36(suppl 2):S231.
11. Leheste JR, Melsen F, Wellner M, et al. Hypocalcemia and osteopathy in mice with kidney-specific megalin gene defect. *FASEB J.* 2003;17:247–249.
12. Melis M, Krenning EP, Bernard BF, et al. Renal uptake and retention of radiolabeled somatostatin, bombesin, neurotensin, minigastrin and CCK analogues: species and gender differences. *Nucl Med Biol.* 2007;34:633–641.
13. Wild D, Behe M, Wicki A, et al. [Lys40(Ahx-DTPA-^{111}In)NH2]exendin-4, a very promising ligand for glucagon-like peptide-1 (GLP-1) receptor targeting. *J Nucl Med.* 2006;47:2025–2033.
14. Hindorf C, Ljungberg M, Strand SE. Evaluation of parameters influencing S values in mouse dosimetry. *J Nucl Med.* 2004;45:1960–1965.
15. Rolleman EJ, Krenning EP, Bernard BF, et al. Long-term toxicity of [^{177}Lu-DOTA^{0},Tyr^{3}]octreotate in rats. *Eur J Nucl Med Mol Imaging.* 2007;34:219–227.
16. Forrer F, Rolleman E, Bijster M, et al. From outside to inside? Dose-dependent renal tubular damage after high-dose peptide receptor radionuclide therapy in rats measured with in vivo ^{99m}Tc-DMSA-SPECT and molecular imaging. *Cancer Biother Radiopharm.* 2007;22:40–49.
17. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys.* 1991;21:109–122.
18. Bitar A, Lisbona A, Bardies M. S-factor calculations for mouse models using Monte-Carlo simulations. *Q J Nucl Med Mol Imaging.* 2007;51:343–351.
19. Stabin MG, Peterson TE, Holburn GE, Emmons MA. Voxel-based mouse and rat models for internal dose calculations. *J Nucl Med.* 2006;47:655–659.
20. De Jong M, Valkema R, Van Gameren A, et al. Inhomogeneous localization of radioactivity in the human kidney after injection of [^{111}In-DTPA]octreotide. *J Nucl Med.* 2004;45:1168–1171.