Dynamic and Static Small-Animal SPECT in Rats for Monitoring Renal Function After 177Lu-Labeled Tyr3-Octreotate Radionuclide Therapy

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High kidney radiation doses during clinical peptide receptor radionuclide therapy (PRRT) with β-particle-emitting radiolabeled somatostatin analogs will lead to renal failure several months after treatment, urging the coinfusion of the cationic amino acids lysine and arginine to reduce the renal radiation dose. In rat PRRT studies, renal protection by the coadministration of lysine was confirmed by histologic examination of kidney specimens indicating nephrotoxicity. In the current study, we investigated dedicated small-animal SPECT/CT renal imaging in rats to monitor renal function in vivo during follow-up of PRRT, with and without lysine. Methods: The following 3 groups of rats were imaged using a multipinhole SPECT/CT camera: controls (group 1) and rats at more than 90 d after therapy with 460 MBq (15 μg) of 177Lu-DOTA-Tyr3-octreotide without (group 2) or with (group 3) a 400-mg/kg lysine coinjection as kidney protection (n ≥ 6 per group). At 90 and 140 d after therapy, static kidney scintigraphy was performed at 2 h after injection of 25 MBq of 99mTc-dimercaptosuccinic acid (99mTc-DMSA). In addition, dynamic dual-isotope renography was performed using 50 MBq of 111In-diethylenetriaminepentaacetic acid (111In-DTPA) and 50 MBq of 99mTc-mercaptoacetyltriglycine (99mTc-MAG3) at 100–120 d after therapy. Results: 111In-DTPA and 99mTc-MAG3 studies revealed a time–activity pattern comparable to those in patients, with a peak at 2–6 min followed by a decline of renal radioactivity. Reduced 111In-DTPA, 99mTc-MAG3, and 99mTc-DMSA uptake indicated renal damage in group 2, whereas group 3 showed only a decrease of 99mTc-MAG3 peak activity. These results indicating nephrotoxicity in group 2 and renal protection in group 3 correlated with levels of urinary protein and serum creatinine and urea and were confirmed by renal histology. Conclusion: Quantitative dynamic dual-isotope imaging using both 111In-DTPA and 99mTc-MAG3 and static 99mTc-DMSA imaging in rats is feasible using small-animal SPECT, enabling longitudinal monitoring of renal function. 99mTc-MAG3 renography, especially, appears to be a more sensitive marker of tubular function after PRRT than serum chemistry or 99mTc-DMSA scintigraphy.
The uptake of 99mTc-radiolabeled dimercaptosuccinic acid (99mTc-DMSA) by the kidneys is directly related to tubular function. Although 99mTc-DMSA scintigraphy is clinically widely used to provide information on renal cortical morphology (19), the exact mechanism of renal handling is unknown. The 2 postulated mechanisms of 99mTc-DMSA uptake are peritubular extraction or absorption of plasma protein–bound 99mTc-DMSA directly from the blood into the proximal tubular cells or glomerular filtration, followed by tubular reabsorption. Discussions on the relative contribution of each of these pathways are ongoing (20–26).

Dynamic imaging of the clearance of radiolabeled diethyleneetriaminepentaacetic acid (DTPA) monitors glomerular filtration, because this small molecule is cleared only via this route. 99mTc-labeled mercaptoacetyltriglycine (99mTc-MAG3) is primarily rapidly extracted from the blood; a 10%–15% fraction is also cleared via glomerular filtration. Extraction of 99mTc-MAG3 by basolateral uptake into cells lining proximal tubules is mediated by an active organic anion transport system, especially in the first convoluted part (S1 and S2) of the proximal tubules (27).

In the current study, dynamic dual-isotope imaging of 111In-DTPA and 99mTc-MAG3 and static 99mTc-DMSA imaging (Fig. 1) were performed to study glomerular filtration, tubular secretion, and peritubular absorption in rats beyond 90 d after therapy with 177Lu-DOTA-Tyr3-octreotate PRRT, with or without kidney protection by lysine coadministration, and results were compared with those for untreated controls. For this purpose, the multipinhole collimated small-animal NanoSPECT/CT camera (Bioscan Inc.) was used (24). Data were correlated with urine and serum chemistry and kidney histology.

MATERIALS AND METHODS

Animals

Animal studies were conducted in accordance with the guidelines of the Animal Welfare Committee of our medical center. For all experiments, male Lewis rats (Harlan) were used (n = 6–9 per group). Body weight (BW) was determined twice a week up to 140 d after therapy. Urine samples were collected every 3 wk after rats were administered PRRT in metabolic cages for 24 h.

Radionuclides, Peptide, Radiopharmaceuticals, and Chemicals

177LuCl3 was obtained from IDB Holland, and DOTA-Tyr3-octreotate was obtained from BioSynthema. Radiolabeling was performed according to previously published procedures (28). A PRRT dose of 460 MBq of 177Lu-DOTA-Tyr3-octreotate was prepared according to the provided procedure. For 99mTc-DMSA and 99mTc-MAG3, the concentrations were 100 and 250 MBq/mL, respectively. 111In-DTPA was prepared by the addition of 100 μL of 4 mM DTPA to 400 MBq of 111InCl3 (pH 3.5–4) and adjusted with saline to a concentration of 250 MBq/mL.

Molecular Imaging

Rats were imaged using a 4-head multipinhole NanoSPECT/CT camera. The energy peak settings (window width ± 10%) were 55, 113, and 208 keV for 177Lu, 171 and 245 keV for 111In, and 140 keV for 99mTc. During 111In and 99mTc dual-isotope imaging, no visible cross-talk from the γ-rays emitted by 111In into the 140-keV window was observed.

Nine pinhole apertures (diameter, 2.5 mm) were used on each camera head, with a transaxial field of view of 60 mm. On the basis of the CT topogram, the axial scan length used for all imaging procedures was 55 mm covering the renal region. Animals were imaged while anesthetized with isoflurane and O2, and body temperature was maintained using a heated bed.

FIGURE 1. Blood enters functional units of kidneys, the nephrons, via afferent arteriole (AA) of renal artery. In the capillary network in glomerulus (G), waste and useful molecules are filtered from blood and transported into proximal tubules (PT). Waste molecules are excreted in primary urine via loop of Henle (LH) and distal tubules (DT) into collecting duct (CD) leading to renal pelvis and bladder. In first convoluted part of PT, megalin/cubilin receptors are expressed, playing a significant role in reabsorption of useful molecules. Extraction from blood in efferent arteriole (EA) (27) directly into PT is another route of excretion of molecules into urine, which is the most prominent way of 99mTc-MAG3 clearance. 111In-DTPA is cleared only via glomerular filtration and is directly excreted into urine, whereas 99mTc-DMSA is partly filtrated in glomeruli, followed by partial reabsorption in PT. 99mTc-DMSA is also partially peritubularly extracted from blood, primarily in last straight part of PT, which may extend into outer medulla of kidney. RV = renal vein.
140 after therapy, 2 h after injection. $^{177}$Lu-DOTA-Tyr<sub>3</sub>-octreotate and $^{99m}$Tc-DMSA images were acquired for 12 min using 24 projections (60 s/projection per head).

Between 100 and 120 d after therapy, dual-isotope dynamic scans were obtained. Before scans were started, rats were anesthetized and a 27-gauge infusion set, with a tubing length of 30 cm (Venisystems; Hospira) filled with saline supplemented with heparin (50 international units [IU]/mL), was inserted into a tail vein. Immediately after the start of the scan, 200 μL (50 MBq) of $^{99m}$Tc-MAG3, followed by 200 μL (50 MBq) of $^{111}$In-DTPA, was administered and flushed with 700 μL of saline (dead volume of catheter, 400 μL). Twenty scans of 2 min each (16 projections per head, 9 s/projection) were obtained, resulting in a total acquisition time of 40 min. To study the influence of extrahydration on the head, 9 s/projection) were obtained, resulting in a total acquisition time of 40 min. To study the influence of extrahydration on the head, 9 s/projection) were obtained, resulting in a total acquisition time of 40 min. To study the influence of extrahydration on the head, 9 s/projection) were obtained, resulting in a total acquisition time of 40 min.

Quantification of the amount of radioactivity in a volume of interest over the kidneys was performed using InVivoScope software (Bioscan). Detected counts in the volume of interest were converted into megabecquerels using a correction factor obtained by scanning a water phantom with the same volume as a rat body to correct for attenuation and filled with a known amount of radioactivity. Radioactivity in the volume of interest over the whole kidneys, or over a cortical part in $^{99m}$Tc-MAG3 images, was quantified in each of the 20 scans during renography to create a time–activity curve. Peak activity over the kidneys was performed using InVivoScope software and at euthanasia at day 140 after therapy blood was collected to store serum.

**Ex Vivo Autoradiography and Histology**

As previously described, the localization of $^{99m}$Tc-DMSA in the kidneys was visualized by ex vivo autoradiography (17). The grade of renal damage was evaluated microscopically according to a scale from grade 0 (no damage) to 1 (little tubular dilation), 2 (basal membrane thickening), 3 (shrinkage of glomeruli, flat tubule epithelium) and 4 (severe tubular and glomerular damage), as described earlier in detail (16).

**Statistics**

Data were expressed as mean ± SD. Statistical analyses were performed using the Student t test.

**RESULTS**

Quantification of $^{177}$Lu in the kidneys at 4 d after therapy showed that 3.8 ± 0.3 MBq were retained in the kidneys of the unprotected animals, whereas 2.0 ± 0.1 MBq $^{177}$Lu localized in the kidneys of the lysine-protected rats (Table 1)—a significant reduction of renal retention of approximately 45%. Irradiation of the renal tubular cells by the β-particles emitted by $^{177}$Lu obviously led to renal problems, starting from 50 d after therapy in PRRT rats without lysine kidney protection. Compared with the normal BW increase over time in controls, BW stabilized in PRRT rats and urinary protein loss was detected. A further increase of protein loss in urine and rise of urea and creatinine serum levels determined at day 90 after therapy confirmed development of renal damage (Table 1). Histologic grading of renal damage was expressed on a scale of 0—4.

### TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days after therapy</th>
<th>Control vs. PRRT</th>
<th>PRRT (P PRRT vs. PRRT + lysine)</th>
<th>PRRT + lysine (P control vs. PRRT + lysine)</th>
</tr>
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<tbody>
<tr>
<td>$^{177}$Lu uptake (MBq/kidney)</td>
<td>4</td>
<td>—</td>
<td>3.8 ± 0.3*</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>BW, % vs. day 0</td>
<td>90</td>
<td>134 ± 3,†</td>
<td>111 ± 6‡</td>
<td>127 ± 5,‡</td>
</tr>
<tr>
<td>140</td>
<td>143 ± 4,‡</td>
<td>113 ± 11,‡</td>
<td>138 ± 6, NS</td>
<td></td>
</tr>
<tr>
<td>Protein in urine (mg/24 h)</td>
<td>100</td>
<td>11 ± 2,‡</td>
<td>62 ± 9,‡</td>
<td>22 ± 8, NS</td>
</tr>
<tr>
<td>Urea in serum (mmol/L)</td>
<td>140</td>
<td>5.3 ± 0.5,‡</td>
<td>32.6 ± 9.4,‡</td>
<td>7.3 ± 0.6, NS</td>
</tr>
<tr>
<td>Creatinine in serum (μmol/L)</td>
<td>140</td>
<td>21 ± 2,‡</td>
<td>60 ± 5,‡</td>
<td>37 ± 3,‡</td>
</tr>
<tr>
<td>Grading histologic renal damage</td>
<td>140</td>
<td>0.4 ± 0.6*, NS</td>
<td>4 ± 0, NS</td>
<td>1.3 ± 0.5, NS</td>
</tr>
<tr>
<td>$^{99m}$Tc-DMSA (%IA/kidney)</td>
<td>90</td>
<td>13.7 ± 1.4*, NS</td>
<td>6.4 ± 2.4,‡</td>
<td>12.5 ± 1.2, NS</td>
</tr>
<tr>
<td>140</td>
<td>18.1 ± 1.9*, NS</td>
<td>5.6 ± 3.2, NS</td>
<td>14.3 ± 1.1, NS</td>
<td></td>
</tr>
<tr>
<td>$^{99m}$Tc-MAG3 (%IA/kidney, 2–6 min)</td>
<td>100–120</td>
<td>13.1 ± 2.6*</td>
<td>4.3 ± 1.5*</td>
<td>8.5 ± 1.6*</td>
</tr>
<tr>
<td>$^{99m}$Tc-MAG3 (%IA/cm&lt;sup&gt;3&lt;/sup&gt; cortex, 2–6 min)</td>
<td>100–120</td>
<td>6.9 ± 1.9*</td>
<td>2.6 ± 1.3*, NS</td>
<td>3.8 ± 1.0*, NS</td>
</tr>
<tr>
<td>$^{111}$In-DTPA (%IA/kidney, 2–4 min)</td>
<td>100–120</td>
<td>4.7 ± 1.0*, NS</td>
<td>2.1 ± 1.4, NS</td>
<td>4.4 ± 0.6, NS</td>
</tr>
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</table>

*P < 0.0001. †P < 0.001. ‡P < 0.05. NS = not significant.

Quantification of retained renal radioactivity was expressed as total MBq/kidney for $^{177}$Lu and %IA/kidney for $^{99m}$Tc-DMSA. Peak activity was expressed as %IA/kidney for $^{111}$In-DTPA and $^{99m}$Tc-MAG3 or as %IA/cm<sup>3</sup> cortex for $^{99m}$Tc-MAG3. BW of rats was expressed as percentage of BW at day 0. Protein loss in urine was expressed in mg/24-h urine. Serum urea and creatinine content was expressed in mmol/L or μmol/L. Histologic grading of renal damage was expressed on a scale of 0–4.
renal damage at day 140 after therapy correlated with these observations. Both massive tubular and glomerular damage were observed in the kidneys of unprotected rats, whereas after lysine coinjection only minor abnormalities in tubular morphology were observed (Table 1).

Tubular damage beyond day 90 after therapy was confirmed by 99mTc-DMSA static imaging (Table 1; Fig. 2). Renal 99mTc-DMSA uptake in nontreated animals ranged from 14% to 19% injected activity (IA) per kidney, which significantly decreased to 2%–8%IA in PRRT-treated rats ($P < 0.0001$) and slightly decreased to 11%–15%IA in the kidneys of rats coinjected with lysine ($P < 0.001$). Ex vivo autoradiography confirmed these data.

Dual-isotope dynamic studies with 111In-DTPA and 99mTc-MAG3 in rats revealed a pattern in the time–activity curves similar to that in humans, with a peak of renal uptake for 111In-DTPA at 2–4 min and for 99mTc-MAG3 at 2–6 min after administration. These peaks were followed by a decline of renal radioactivity, until most radioactivity was excreted at 20 min after injection and stabilized until 40 min after injection (Table 1; Fig. 3). Quantification of total renal 99mTc-MAG3 radioactivity showed a mean peak activity of 13.1% IA in nontreated rats, whereas in PRRT rats only 4.3%IA was detected. Lysine coadministration protected 99mTc-MAG3 extraction capacity of tubular cells after PRRT, because a significantly higher uptake ($P < 0.0001$) of 8.5%IA renal radioactivity was quantified in the PRRT + lysine group. Mean peak activities quantified only in cortical regions of the kidneys of the control, PRRT, and PRRT + lysine rats were 6.9%, 2.6%, and 3.8%IA/cm³ cortex, respectively.

111In-DTPA imaging revealed a small but insignificant difference in peak activity between the control and PRRT + lysine groups (4.7% vs. 4.4%IA, respectively); however, 111In-DTPA uptake in the PRRT-only group was significantly less (2.1%IA, $P = 0.0003$), indicating that glomerular filtration was impaired after PRRT without kidney protection.

In clinical renography studies, patients are hydrated (i.e., 500 mL water by mouth 1 h before imaging) but are imaged with an empty bladder to stimulate efflux of radioactivity from the renal pelvis. Therefore, an extra group of control rats was imaged after extra hydration before the scan. The mean peak activities of 99mTc-MAG3 and 111In-DTPA remained unaltered, whereas the excretion of 111In-DTPA was unchanged and the efflux of 99mTc-MAG3 was moderately enhanced during the last phase between 20 and 40 min after injection (data not shown).

DISCUSSION

In this small-animal SPECT study in rats, noninvasive renal imaging using 99mTc-DMSA, 99mTc-MAG3, and 111In-DTPA provided insight into both tubular and glomerular functions after high absorbed kidney radiation doses to monitor renal function as follow-up after PRRT. Simultaneous renography using dual-isotope dynamic imaging with 99mTc-MAG3 and 111In-DTPA was advantageous in restricting animal discomfort. Accurate quantification of renal radioactivity was obtained in short scans when 50 MBq of both 99mTc-MAG3 and 111In-DTPA were administered, leading to activity levels of 0.6–6 MBq per kidney for 99mTc and 0.4–2.5 MBq for 111In. Compared with clinical

**FIGURE 2.** Typical images of maximum-intensity projections and coronal slices of kidney after 99mTc-DMSA scintigraphy performed at day 140 after therapy in nontreated control, PRRT–treated, or PRRT + lysine–treated rats. Ex vivo autoradiograms are of frozen kidney sections, prepared immediately after imaging. MIP = maximum-intensity projection. Lys = lysine.
renography using frames of 15 s during a 20-min scan, the 2-min frames in the rats were relatively long but had to be chosen because of the rotation time of the camera heads between projections. Recently, Bioscan developed high-sensitivity apertures with enlarged pinhole diameters, offering improved time resolution. In the Linoview study (26), continuous dynamic SPECT was performed using 15-s frames for 30 min after injection of 99mTc-MAG3, and the newly developed U-SPECT dedicated small-animal SPECT camera (Milabs) also will enable the acquisition of shorter frames than were used in this study (29).

Renographic 99mTc-MAG3 studies demonstrated more efficient elimination of the radioactivity from the kidneys in conscious mice than in anesthetized ones, indicating that the metabolic state of the animals is a confounding factor during dynamic renal imaging (26). Although the clearance rate of 99mTc-MAG3 and 111In-DTPA in our current study in anesthetized rats might not reflect the true physiologic state, the 3 groups of rats to be compared were imaged under equal, controlled conditions. Furthermore, the efflux of the radiopharmaceuticals from the kidneys to the bladder could be hampered by a filled bladder. The efflux of 99mTc-MAG3 during the last phase of clearance was only slightly improved by extrahydration of the rats at 45 min before imaging (to stimulate emptying of the bladder), whereas the peak uptake of 99mTc-MAG3 and 111In-DTPA remained unchanged. Because of these extrahydration findings, hydration of the rats was not considered essential.

Previous experiments demonstrated that approximately 3% of the radiopeptide is reabsorbed per kidney (16,17)—a process that is mediated by the multiligand scavenger receptor megalin, which is expressed in the first part of the cortical proximal tubules (30,31). Competition for the megalin receptor by lysine and radiolabeled octreotate, containing 1 lysine residue, might explain the reduction of renal retention found after coadministration with cationic amino acids (10,13,31). In the current study, PRRT was administered using 460 MBq of 177Lu-DOTA-Tyr3-octreotate. Long-term nephrotoxic effects were similar to those after the administration of 555 MBq of PRRT, although the reduction of 99mTc-DMSA uptake was less severe (17). The aberrant localization pattern in PRRT kidney 99mTc-DMSA autoradiograms (Fig. 2) demonstrated that 99mTc-DMSA was retained only in the nonmegalin receptor–expressing S3 part of the proximal tubules, located in the outer medulla, in contrast to the localization in control kidneys. Because 111In-DTPA renography demonstrated poor glomerular filtration in PRRT rats, 99mTc-DMSA will not or will hardly be filtered and therefore not be reabsorbed in the proximal tubules. Therefore, peritubular extraction was the only route of 99mTc-DMSA retention in this part of the proximal tubules, as illustrated by the radioactivity localized in the

**FIGURE 3.** (A and B) Renography after injection of 50 MBq of 99mTc-MAG3, expressed as %IA/kidney (A) and %IA/cm³ cortex (B). (C) Renography after injection of 50 MBq of 111In-DTPA, expressed as %IA/kidney. (D) Typical example of a maximum-intensity-projection dual-isotope image in control rats, 6 min after injection: 99mTc-MAG3 (left), 111In-DTPA (middle), and merged (right) images. Lys = lysine.
outper mediated damage in the first convoluted part of the cortical proximal tubules as demonstrated by the unimpaired 99mTc-DMSA localization pattern. A role of megalin in the tubular reabsorption of 99mTc-DMSA after glomerular filtration was confirmed by our observation that only 35%–55% of 99mTc-DMSA renal uptake was found when we imaged kidney-specific megalin-deficient mice (32), compared with wild-type mice (data not shown). Also in Clcn5 knockout mice, a model of Dent disease showing defective megalin- and cubilin-mediated endocytosis, heavily reduced 99mTc-DMSA uptake was observed, which matched results described in studies in patients with Dent disease or Fanconi syndrome (25,26,33). Therefore, the conclusion drawn by Müller-Suur et al. that only peritubular extraction was responsible for renal 99mTc-DMSA uptake has been contradicted by these observations (22).

99mTc-MAG3 imaging showed no peak of tubular extraction in PRRT rats, either after total renal or after cortex-only 99mTc quantification; the latter method avoided confounding radioactivity already excreted into the renal pelvis (Fig. 3B). The clearance of 99mTc-MAG3 is not megalin or cubulin receptor–dependent, as discovered in Clcn5 knockout mice (26) and confirmed by us in megalin-deficient mice (data not shown), but is mediated by organic anion transport. This transport system is expressed in cells at the basolateral side of the convoluted proximal tubules and appeared to be inactive beyond 100 d after PRRT. Although lysine coadministration during PRRT significantly protects kidney function, 99mTc-MAG3 tubular extraction was clearly impaired when compared with control rats. It was proposed that 99mTc-DMSA was the optimal tracer to monitor and predict renal function (3,34). However, the current data suggest that 99mTc-MAG3 renography is a more sensitive method to quantify loss of function of cortical proximal tubules than 99mTc-DMSA scintigraphy after PRRT. Unaffected 99mTc-MAG3 renography is one of the inclusion criteria before 99mTc- or 177Lu-based PRRT and served, which matched results described in studies in patients with PRRT. Unaffected 99mTc-MAG3 renography is one of the most sensitive markers of tubular function after PRRT than 99mTc-DMSA imaging, serum urea urinary protein content, or histology. Therefore, 99mTc-MAG3 scintigraphy might gain a more prominent role in clinical PRRT.

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