Exendin-4–Based Radiopharmaceuticals for Glucagonlike Peptide-1 Receptor PET/CT and SPECT/CT

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Strong overexpression of glucagonlike peptide-1 (GLP-1) receptors in human insulinomas provides an attractive target for imaging. The first clinical trials demonstrated that GLP-1 receptor SPECT/CT using [Lys40(Ahx-[6-aminohexanoic acid]-DOTA-111In)NH2]-exendin-4 can localize hardly detectable insulinomas. However, [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 imaging has drawbacks related to the use of 111In in that it is costly and carries a relatively high radiation burden for the patient. The aim of this study was the preclinical evaluation of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 for PET/CT and [Lys40(Ahx-hydrazinonicotinamide [HYNIC]-99mTc)NH2]-exendin-4 for SPECT/CT. Methods: Internalization, biodistribution, dosimetry, and imaging studies were performed in the Rip1Tag2 mouse model of pancreatic β-cell carcinogenesis and compared with our gold standard [Lys40(Ahx-DOTA-111In)NH2]-exendin-4. Poly-glutamic acid and Gelofusine, a gelatin-based plasma expander, were used for renal uptake reduction studies. Results: The tumor uptake of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 was 205 ± 59 percentage injected activity per gram of tissue at 4 h. Other GLP-1 receptor–positive organs showed more than 4.8 times lower radioactivity uptake. [Lys40(Ahx-HYNIC-99mTc/ethylenediaminediacetic acid [EDDA])NH2]-exendin-4, compared with its 111In- and 68Ga-labeled sister compounds, showed significantly less tumor and organ uptake. The significantly lower tumor and organ uptake of [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 did not result in inferior tumor-to-organ ratios or reduced image quality. All radiopeptides tested showed a high tumor-to-background ratio, resulting in the visualization of small tumors (maximum diameter between 1.0 and 3.2 mm) by SPECT and PET. The only exception was the kidneys, which also showed high uptake. This uptake could be reduced by 49%–78% using poly-glutamic acid, Gelofusine, or a combination of the 2. The estimated effective radiation dose was 3.7 μSv/MBq for [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4, which was 8 times less than that for [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 and 43 times less than that for [Lys40(Ahx-DOTA-111In)NH2]-exendin-4. Conclusion: These promising pharmacokinetic and imaging data show that [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 and [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 are suitable candidates for clinical GLP-1 receptor imaging studies.

Key Words: glucagon-like peptide-1 receptor; insulinoma; exendin-4; 68Ga; 99mTc

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Over the last 15 y, peptide receptor targeting of cancer cells with radiolabeled peptides has become an important method for the diagnosis and treatment of cancer patients (1,2).

The high-density distribution of membrane peptide receptors in many different tumors, as shown on in vitro autoradiographic studies, represents the molecular basis of this application (1). [111In-diethylenetriaminepentaacetic acid (DTPA)9]-octreotide (OctreoScan; Covidiens) was the first probe for somatostatin receptor subtype 2 targeting to become an integral part of the routine diagnostic work-up of patients with gastroenteropancreatic neuroendocrine tumors (3). Especially in gut carcinoid tumors and gastrinomas, [111In-DTPA9]-octreotide proved superior to conventional imaging methods such as sonography, CT, and MRI (4–6). In insulinomas, the sensitivity of [111In-DTPA9]-octreotide is below 50% (7) because somatostatin receptor subtype 2 is expressed by less than 60% of insulinomas (8). The conventional imaging methods also have limited sensitivity because of the small size of insulinomas (9,10). The amine precursor 6,18F-fluoro-L-dopa shows controversial results, with sensitivities ranging from 17% up to 90% (11,12). Only angiography in combination with intraarterial calcium stimulation and
venous sampling has been shown to improve the sensitivity, but this is an invasive procedure with a concomitant risk of complications (13). Thus, there is a clear need for a method that improves preoperative insulinoma imaging, especially in view of the fact that preoperative localization facilitates surgery for insulinoma, which is the only curative treatment option (10,14).

A promising new approach is the targeting of glucagon-like peptide-1 (GLP-1) receptors because their high densities in insulinoma provide an attractive target for imaging using GLP-1 receptor–avid radioligands. Especially in benign insulinoma, the GLP-1 receptor density is high, with almost 100% incidence (8). Consequently, GLP-1 receptor–avid radioligands have been developed and evaluated (15,16). Preclinical animal studies have shown the ability of [Lys40(Ahx [6-aminohexanoic acid]-DTPA-111In)NH2]-exendin-4 to successfully localize small insulinomas in the Rip1Tag2 mouse tumor model (17). Treatment studies in the same animal tumor model have shown the potential of GLP-1 receptor targeting as a therapeutic approach (18). Most important, the first trials in patients showed promising results in the noninvasive localization of insulinomas (19). In 6 of 6 patients, [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 SPECT/CT successfully detected pancreatic and ectopic insulinomas, which had previously not been identified with certainty using conventional methods (20). However, [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 has several drawbacks related to the use of 111In, resulting in a relatively high radiation burden for the patient. A 99mTc-labeled GLP-1 receptor analog may overcome these drawbacks, and a PET tracer may have advantages over conventional GLP-1 receptor imaging with 111In.

The aim of this study was the preclinical evaluation of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 for PET/CT and [Lys40(Ahx-hydrazinonicotinamide [HYNIC].99mTc(ethylenediaminediacetic acid [EDDA])NH2]-exendin-4 for SPECT/CT. Internalization, biodistribution, imaging, and dosimetry studies were performed and compared with our gold standard, [Lys40(Ahx-DOTA-111In)NH2]-exendin-4. Most in vivo studies were performed in transgenic Rip1-Tag2 mice, which is an animal model suitable for in vivo GLP-1 receptor targeting (17).

**MATERIALS AND METHODS**

Abbreviations of the common amino acids are in accordance with the recommendations of the Commission of Biochemical Nomenclature of the International Union of Pure and Applied Chemistry—International Union of Biochemistry (21).

**Reagents and Instrumentation**

[Lys40(Ahx-DOTA)NH2]-exendin-4 and [Lys40(Ahx-HYNIC)NH2]-exendin-4 were custom-synthesized by Anawa Trading SA and Peptide Specialty Laboratories GmbH, respectively. Matrix-assisted laser desorption ionization—mass spectrometry measurements were done on a Voyager sSTR equipped with an Nd:YAG laser (355 nm) (Applied BioSystems). 67GaCl3, 111InCl3, and the 99Mo/99mTc generator were obtained from Coviden. The 68Ga/67Ga generator was delivered by Eckert and Ziegler. Analytic reversed-phase high-performance liquid chromatography (HPLC) was performed on a Bischof HPLC system (Metrohm AG) with HPLC pumps (model 2250) and a λ-1010 ultraviolet detector (Metrohm AG), as described elsewhere (17).

Dulbecco’s modified Eagle’s medium (high glucose, pH 7.4) supplemented with 10% fetal bovine serum, 2% l-glutamine, and 1% penicillin-streptomycin from Gibco BRL was used. C57BL/6J mice and transgenic Rip1Tag2 mice were scanned and analyzed either with an inline PET/CT system (Discovery STE; GE Healthcare) or with an MRI scanner (Magnemot Expert; Siemens) and a SPECT scanner (e.cam SPECT scanner; Siemens), which was modified with a multipinhole aperture (22). SPECT images were reconstructed using a HiSPECT reconstruction program (SciVis). SPECT and MR images were manually fused on a Hermes workstation (Hermes Medical Solutions). All dosimetric calculations were performed using the OLINDA/EXM 1.0 software (Vanderbilt University, 2003) (23).

**Radiolabeling of Peptides**

[Lys40(Ahx-DOTA)NH2]-exendin-4 with 111InCl3 was radiolabeled as previously described (17). The radiolabeled solution was then subjected to quality control by analytic reversed-phase HPLC.

[Lys40(Ahx-DOTA)NH2]-exendin-4 was radiolabeled with 67Ga as described for 111InCl3. An aliquot of 40 μL (0.2 mmol/L, 40 μg) of peptide was dissolved in 200 μL of sodium acetate buffer (0.4 mol/L, pH 5.0) before 23 MBq of 67GaCl3 were added. 68Ga was eluted from a commercially available generator according to the method of Zhernovosev et al. (24). Afterward, the eluate was purified of 68Ge(IV), Zn(II), Ti(IV), and Fe(III) using a 50W-X8 cation exchanger chromatographic column (BioRad) (<400 mesh) and 80% acetone/0.15N hydrochloric acid. After elution from the exchanger column (400 μL of 97.6% acetone/0.05N HCl solution), 99mTc(III) was incubated with 50 μg of [Lys40(Ahx-DOTA)NH2]-exendin-4 in a 0.25 M N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) solution (400 μL; pH 6.3-3.9) for 30 min at 95°C using a microwave oven (Biotage).

The radiolabeling of [Lys40(Ahx-HYNIC)NH2]-exendin-4 with 99mTc followed a 2-vial kit formulation. One milliliter of a solution containing 15 mg (84 μmol) of tricine, 35 μg of [Lys40(Ahx-HYNIC)NH2]-exendin-4, and 40 μg of SnCl2 (10 μL of 22.2 mM SnCl2·H2O in 0.1 M HCl) was filtered into a glass vial strictly under air protection. One-half milliliter of a solution containing 5 mg of EDDA (pH was adjusted to 7 with 1 M NaOH) was filtered into a second glass vial. The glass vials were immediately frozen in liquid nitrogen, lyophilized, and closed afterward under a vacuum. For labeling, the EDDA vial was reconstituted with 0.5 mL of saline and added to the [Lys40(Ahx-HYNIC)NH2]-exendin-4 vial, followed by 370 MBq of 99mTcO4−, and incubated for 10 min at 95°C. After cooling to room temperature, the reaction mixture was mixed by HPLC.

**Cell Culture, Radioligand Internalization, and Externalization Studies**

GLP-1 receptor–expressing β-tumor cells were established from β-cell tumors of Rip1Tag2 mice, as described previously (17). For internalization experiments, the cells were seeded at a density of 0.8–1 million cells per well in 6-well plates and incubated overnight with internalization buffer. Afterward, 0.25 pmol of the respective radiopeptide was added to the medium...
(final concentration, 0.17 nmol/L) and incubated at 37°C. To determine nonspecific membrane binding and internalization, a large excess of unlabeled peptide was used in selected wells. The internalization was stopped at appropriate time points (30 min and 1, 2, and 4 h) by removing the medium, and the cells were treated as described previously (17).

For externalization studies, β-tumor cells (0.8–1 million) were incubated with 0.25 pmol of radiopeptide (0.17 nmol/L) for 120 min. Then the medium was removed, and the cells were treated as described previously (17). All in vitro experiments were performed twice (triplicates in each experiment).

Animal Model

Animals were maintained and treated in compliance with the guidelines of the Swiss regulations (approval 789). Male and female Rip1Tag2 transgenic mice and female wild-type mice (C57BL/6J mice) were used for biodistribution studies, pinhole SPECT/MRI, and PET/CT. Transgenic Rip1Tag2 mice developed β-cell tumors in the pancreas in a multistage tumorigenesis pathway. These tumors were characterized by a high expression of GLP-1 receptors (17). Phenotypic and genotypic analyses of Rip1Tag2 mice have been described previously (25). At intervention, all mice were between 11 and 13 wk old.

Biodistribution in Rip1Tag2 Mice

Ten picomoles (70–110 kBq) of the respective radiopeptide diluted in 100 µL of a 1% human albumin solution were injected into the tail vein of Rip1Tag2 mice. Rip1Tag2 mice were sacrificed at the following time points after injection (n = 3–6 per time point): For [Lys⁴⁰(Ahx-DOTA-¹¹¹In)NH₂]-exendin-4, mice were sacrificed at 1, 4, 12, 36, 84, and 168 h; for [Lys⁴⁰(Ahx-DOTA-⁶⁸Ga)NH₂]-exendin-4, at 0.5, 1, 2, and 4 h; and for [Lys⁴⁰(Ahx-HYNIC-⁶⁸Ga)NH₂]-exendin-4, at 0.5, 2, 4, and 18 h. Organs, blood, and tumors were collected, and the radioactivity was measured in a γ-counter. The radioactivity uptake in organs and tumors was calculated as percentages of injected activity per gram of tissue (%IA/g) and percentage of injected activity per organ (%IA/organ).

To determine the nonspecific uptake of the respective radiopeptide, Rip1Tag2 mice were coinjected with 10 pmol of the radiolabeled peptide and 5 nmol of the respective nonlabeled peptide and sacrificed 4 h later.

Radiation Dose Calculation

Mice biodistribution data were used to generate the residence time for each radiopeptide. Because of the absence of specific activity accumulation in bones and red marrow (18), a linear relationship between the blood residence time and red marrow residence time was assumed to estimate the red marrow radiation dose (26). The proportionality factor was the ratio between the red marrow mass and the blood mass in humans.

OLINDA/EXM was used to integrate the fitted time–activity curves. Organ and effective doses were estimated with OLINDA/EXM using the whole-body adult female model and the weighting factors recommended by the International Commission on Radiological Protection (27). For all calculations, the assumption was made that the Rip1Tag2 mouse biodistribution, determined as the %IA/organ, was the same as the human biodistribution.

GLP-1 Receptor Imaging with Multipinhole SPECT/MRI

Two Rip1Tag2 mice were injected with 37 MBq of [Lys⁴⁰(Ahx-HYNIC-⁹⁹mTc/EDDA)NH₂]-exendin-4 into the tail vein. Four hours after injection, multipinhole SPECT images of both Rip1Tag2 mice (under isoflurane anesthesia and lying prone) were obtained (22). Images were acquired from 60 angles, with a minimum of 30 kilo counts per angle, resulting in a scan time of 60 min. Shortly thereafter, Rip1Tag2 mice were scanned in an MRI scanner in the same position. To enhance the signal-to-noise ratio, a specially designed and modified small-animal saddle coil was used. Coronal high-resolution slices were obtained using a 3-dimensional (3D) double-echo-in-steady-state sequence. Transverse slices were reconstructed from the 3D dataset to obtain slices for image fusion. Reconstructed transverse MR and SPECT images were manually fused using the anatomic information obtained from both imaging modalities. After imaging, necropsy was performed in both animals, and the size of tumors was measured.

GLP-1 Receptor Imaging with PET/CT

One Rip1Tag2 mouse was injected with 10 pmol (130 kBq) of [Lys⁴⁰(Ahx-DOTA-⁶⁸Ga)NH₂]-exendin-4 as described above. Sixty minutes after injection, the mouse was sacrificed and bilateral nephrectomy was performed. PET images (3D mode) of the mouse lying prone were obtained for 1 h with a PET/CT hybrid scanner. The image matrix was 256 × 256, and images were reconstructed as 1-mm-thick sections using an iterative algorithm. The CT data from the PET/CT examination were reconstructed in the transverse plane as 1-mm-thick sections. The following parameters were used for imaging: 130 kV, 80 mA/s, 1.5 s per rotation, and 1 mm/s table speed.

Renal Uptake Reduction Studies

For renal uptake reduction studies, 80 mg (3–15 kD) of L-polyglutamic acid (PGA; Sigma-Aldrich) per milliliter or 40 mg of Gelofusine (Braun) per milliliter were dissolved in saline as described previously (28). Rip1Tag2 mice were injected intravenously with 100 µL of one solution or 200 µL of both solutions just before intravenous administration of 10 pmol of

| TABLE 1. Comparison of Internalization Kinetics for ¹¹¹In-, ⁶⁷Ga-, and ⁹⁹mTc-Labeled Exendin-4 in β-Tumor Cells |
|-----------------|--------|--------|--------|--------|
| **Compound**    | 0.5 h  | 1 h    | 2 h    | 4 h    |
| [Lys⁴⁰(Ahx-DOTA-¹¹¹In)NH₂]-exendin-4 | 1.03 ± 0.14 | 2.03 ± 0.18 | 4.97 ± 0.4 | 9.75 ± 0.65 |
| [Lys⁴⁰(Ahx-DOTA-⁶⁸Ga)NH₂]-exendin-4 | 1.22 ± 0.08 | 2.48 ± 0.29 | 5.10 ± 0.26 | 10.35 ± 0.43 |
| [Lys⁴⁰(Ahx-HYNIC-⁹⁹mTc/EDDA)NH₂]-exendin-4 | 0.73 ± 0.29 | 1.50 ± 0.49 | 3.37 ± 1.27 | 8.50 ± 1.94 |
| 1-way ANOVA | P = 0.002 | P = 0.001 | P = 0.003 | P = 0.0001 |

Values and SD are result of 2 independent experiments (triplicates in each experiment) and are expressed as specific internalization (% added radioactivity/10⁶ cells ± SD). Significance was analyzed by 1-way ANOVA.

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Graphing and curve fitting were performed using Microcal Origin. Analyzed in Microsoft Excel using linear regression analysis.

Radioactivity uptake in organs and tumors was calculated as animal and 1 PGA plus Gelofusine–treated animal underwent Tag2 mice (1062).

Table 2 shows the biodistribution in Rip1Tag2 mice at 0.5, 1, 2, and 4 hours after injection of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4. One hour later, Rip1-Tag2 mice (n = 3 per cohort) were sacrificed. One control animal and 1 PGA plus Gelofusine–treated animal underwent PET/CT just before tumor and organ collection. Finally, the radioactivity uptake in organs and tumors was calculated as %IA/g and %IA/organ.

### Statistical Analysis

The calculation of means and SDs for internalization and biodistribution was performed in Excel (Microsoft). The correlation between the rate of internalization and tumor or lung uptake was analyzed in Microsoft Excel using linear regression analysis. Graphing and curve fitting were performed using Microcal Origin. One-way ANOVA for groups, including Tukey’s posttest for pairwise comparison, was performed using Palaeontological Statistics software. P values less than 0.05 were considered significant.

Table 3 shows the biodistribution in Rip1Tag2 mice at 0.5, 2, 4, and 18 hours after injection of [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4.

### Results

#### Synthesis and Radiolabeling

DOTA and HYNIC were coupled via the Lys side chain of the C-terminally extended exendin-4 using Ahx as a spacer. The composition and structural identity of [Lys40(Ahx-DOTA)NH2]-exendin-4 and [Lys40(Ahx-HYNIC)NH2]-exendin-4 were verified by analytic HPLC and matrix-assisted laser desorption ionization–mass spectrometry ([Lys40(Ahx-DOTA)NH2]-exendin-4: 4,815.21 [M+H+] and [Lys40(Ahx-HYNIC)NH2]-exendin-4: 4,563.78 [M+H+]). The labeling yield of [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 and [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 was 98% and 95% at a specific activity of 19 GBq/μmol and 48 GBq/μmol, respectively. [Lys40(Ahx-DOTA-68Ga)NH2]-

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**Table 2. Biodistribution in Rip1Tag2 Mice at 0.5, 1, 2, and 4 Hours After Injection of [Lys40(Ahx-DOTA-68Ga)NH2]-Exendin-4**

<table>
<thead>
<tr>
<th>Organ</th>
<th>0.5 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs*</td>
<td>40.8 ± 3.5</td>
<td>42.5 ± 5.1</td>
<td>31.4 ± 2.9</td>
<td>42.5 ± 5.1</td>
</tr>
<tr>
<td>Pancreas*</td>
<td>17.0 ± 2.4</td>
<td>13.5 ± 4.4</td>
<td>16.8 ± 6.3</td>
<td>13.5 ± 1.0</td>
</tr>
<tr>
<td>Stomach*</td>
<td>4.05 ± 0.33</td>
<td>4.08 ± 0.59</td>
<td>2.56 ± 0.35</td>
<td>2.14 ± 0.77</td>
</tr>
<tr>
<td>Tumor*</td>
<td>185 ± 33</td>
<td>209 ± 44</td>
<td>207 ± 60</td>
<td>205 ± 59</td>
</tr>
<tr>
<td>Kidneys</td>
<td>255 ± 14</td>
<td>230 ± 33</td>
<td>252 ± 24</td>
<td>202 ± 34</td>
</tr>
<tr>
<td>Liver</td>
<td>0.88 ± 0.04</td>
<td>0.61 ± 0.11</td>
<td>0.63 ± 0.12</td>
<td>0.61 ± 0.11</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.14 ± 0.12</td>
<td>1.91 ± 0.50</td>
<td>2.10 ± 0.73</td>
<td>2.28 ± 0.59</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.30 ± 0.10</td>
<td>1.13 ± 0.51</td>
<td>0.97 ± 0.09</td>
<td>1.00 ± 1.03</td>
</tr>
<tr>
<td>Bone</td>
<td>1.03 ± 0.33</td>
<td>1.01 ± 0.91</td>
<td>1.07 ± 0.13</td>
<td>0.89 ± 0.52</td>
</tr>
<tr>
<td>Blood</td>
<td>2.08 ± 0.49</td>
<td>1.35 ± 0.17</td>
<td>0.49 ± 0.03</td>
<td>0.29 ± 0.10</td>
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<tr>
<td>Tumor/blood</td>
<td>88.9</td>
<td>155</td>
<td>423</td>
<td>706</td>
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<tr>
<td>Tumor/muscle</td>
<td>142</td>
<td>185</td>
<td>214</td>
<td>205</td>
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<tr>
<td>Tumor/pancreas</td>
<td>10.9</td>
<td>15.5</td>
<td>12.4</td>
<td>15.2</td>
</tr>
<tr>
<td>Tumor/lungs</td>
<td>4.52</td>
<td>4.91</td>
<td>6.60</td>
<td>4.82</td>
</tr>
<tr>
<td>Tumor/kidneys</td>
<td>0.72</td>
<td>0.91</td>
<td>0.82</td>
<td>1.01</td>
</tr>
</tbody>
</table>

*GLP-1 receptor–positive organs.
Results are expressed as %IA/g (mean ± SD), n ≥ 3.

<table>
<thead>
<tr>
<th>Organ</th>
<th>0.5 h</th>
<th>2 h</th>
<th>18 h</th>
</tr>
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<tbody>
<tr>
<td>Lungs*</td>
<td>14.6 ± 4.3</td>
<td>18.5 ± 5.9</td>
<td>15.9 ± 5.6</td>
</tr>
<tr>
<td>Pancreas*</td>
<td>7.1 ± 2.0</td>
<td>9.6 ± 1.5</td>
<td>7.4 ± 2.2</td>
</tr>
<tr>
<td>Stomach*</td>
<td>1.18 ± 0.32</td>
<td>1.36 ± 0.32</td>
<td>1.20 ± 0.30</td>
</tr>
<tr>
<td>Tumor*</td>
<td>67 ± 13</td>
<td>98 ± 19</td>
<td>93 ± 20</td>
</tr>
<tr>
<td>Kidneys</td>
<td>63 ± 10</td>
<td>57 ± 14</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>Liver</td>
<td>0.83 ± 0.20</td>
<td>0.71 ± 0.28</td>
<td>0.72 ± 0.20</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.59 ± 0.10</td>
<td>0.47 ± 0.17</td>
<td>0.52 ± 0.10</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.16 ± 0.08</td>
<td>0.13 ± 0.10</td>
<td>0.17 ± 0.08</td>
</tr>
<tr>
<td>Bone</td>
<td>0.19 ± 0.03</td>
<td>0.16 ± 0.03</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>Blood</td>
<td>2.34 ± 0.47</td>
<td>0.55 ± 0.11</td>
<td>0.35 ± 0.13</td>
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<tr>
<td>Tumor/blood</td>
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<td>177</td>
<td>266</td>
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<td>754</td>
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<td>10.2</td>
<td>12.6</td>
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<td>Tumor/lungs</td>
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<td>5.29</td>
<td>5.86</td>
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<td>Tumor/kidneys</td>
<td>1.06</td>
<td>1.72</td>
<td>1.55</td>
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</table>

*GLP-1 receptor–positive organs.
Results are expressed as %IA/g (mean ± SD), n ≥ 3.
exendin-4 was radiolabeled using microwave heating with a labeling yield of greater than 98% at a specific activity of 13 GBq/μmol.

**In Vitro Internalization and Externalization Studies**

Table 1 shows the results of specific internalization of GLP-1 receptor agonists into β-tumor cells. Between 80% and 99% of totally internalized radioligands were specifically internalized. The rate of internalization was not significantly different between the 111In- and the 67Ga-labeled exendin-4. A significant difference was found between 99mTc- and 111In- and between 99mTc- and 67Ga-labeled exendin-4.

The kinetics of externalization were studied with β-tumor cells exposed to the radioligand for 2 h at 37°C. Within 4 h, only 18.7%–23% of the radioligands were released from the β-tumor cells and the externalization leveled off (not shown). There was no significant difference in the rate of externalization among the 3 radiopeptides.

**Animal Biodistribution Studies**

We have shown previously that the tumor uptake of [Lys40(Ahx-DTPA-111In)NH2]-exendin-4 is peptide amount-dependent, with the highest uptake at a peptide mass of 10 pmol (18). As a result, 10 pmol of radiopeptide was used in all biodistribution experiments. Biodistribution data and tumor-to-tissue ratios of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 and [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 are summarized in Tables 2 and 3, respectively. Both radiopeptides showed a fast blood clearance and high tumor-to-normal organ ratios already at early time points (Tables 2 and 3). One-way ANOVA showed significant differences among exendin-4 analogs (Table 4). [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 showed the lowest and [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 the highest tumor and organ uptake. Tukey’s posttest for individual comparison showed no difference in tumor uptake between [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 and [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4. The accumulated activity in GLP-1 receptor–positive organs such as lung and pancreas was high, at 42.5 ± 5.1 %IA/g and 13.5 ± 1.0 %IA/g 4 h after injection of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4. The highest uptake was found in the tumor and kidneys at 4 h after injection of the 111In- and 68Ga-labeled DOTA conjugate. Blocking with a 500-times excess of the respective cold ligand reduced the tumor uptake of all tested radiopeptides by more than 94%, whereas the kidney uptake was not affected.
An important value for the diagnostic use of GLP-1 receptor tracers is the tumor-to-pancreas ratio, which was between 12 and 15.2 for all radiopeptides tested.

**Dosimetry**

Table 5 shows the radiation dose estimation extrapolated to humans after injection of $^{111}$In-, $^{68}$Ga-, and $^{99}$mTc-labeled exendin-4. Bio-distribution data expressed as %IA/organ (Supplemental Tables 1–3; supplemental materials are available online only at http://jnm.snmjournals.org) were used to generate the residence time for each radiopeptide. The estimated effective radiation dose is 0.16 mSv/MBq for $[^{111}\text{In}](\text{Ahx-DOTA})$-exendin-4, 0.032 mSv/MBq for $[^{68}\text{Ga}](\text{Ahx-DOTA})$-exendin-4, and only 0.0037 mSv/MBq for $[^{99}\text{mTc}](\text{HYNIC})$-exendin-4. The highest radiation dose was calculated for the kidneys.

**In Vivo GLP-1 Receptor Imaging**

Figure 1 shows the iteratively reconstructed multipinhole SPECT/MR images of 1 Rip1Tag2 mouse at 4 h after injection of 37 MBq of $[^{99}\text{mTc}](\text{HYNIC})$-exendin-4. In contrast to high-resolution MRI, pinhole $[^{99}\text{mTc}](\text{HYNIC})$-exendin-4 SPECT visualized 4 small insulinomas with a diameter between 1.0 and 3.2 mm. Other GLP-1 receptor–positive organs, such as the lung and pancreas, were hardly visible.
Figure 2 shows PET/CT images of 1 Rip1Tag2 mouse at 1 h after injection of 130 kBq of [Lys 40(Ahx-DOTA-68Ga)NH2]-exendin-4. Before imaging, bilateral nephrectomy was performed because tumor delineation from the kidneys was not possible before nephrectomy. After nephrectomy, PET/CT images demonstrated impressive focal uptake in 2 tumors in the pancreatic body after injection of only 130 kBq of [Lys 40(Ahx-DOTA-68Ga)NH2]-exendin-4 (Figs. 2C and 2D). The maximal diameter of these tumors was only 2.3 and 1.5 mm.

Renal Uptake Reduction Studies

Figures 3A and 3B show kidney and tumor uptake of [Lys 40(Ahx-DOTA-68Ga)NH2]-exendin-4 after pretreatment with PGA or Gelofusine. The uptake in tissues other than kidneys did not differ significantly between the control group and the kidney protection group. In comparison with the control group, PGA and Gelofusine showed a kidney uptake reduction of 49% and 60%, respectively ($P < 0.01$). The combination of PGA and Gelofusine was even more effective than PGA alone, with a kidney uptake reduction of 78% ($P = 0.0002$). The tumor-to-kidney ratio was 4.9 after PGA plus Gelofusine treatment and 2.4 after Gelofusine treatment. However, no significant difference in kidney protection was found between Gelofusine alone and the combination of PGA and Gelofusine ($P = 0.055$). Figures 3C and 3D show [Lys 40(Ahx-DOTA-68Ga)NH2]-exendin-4 PET/CT scans of 1 animal with kidney protection and 1 animal without kidney protection.
DISCUSSION

GLP-1 receptor imaging is a novel approach for preoperative localization of insulinoma. First clinical studies using 111In-labeled GLP-1 receptor agonist [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 detected 6 of 6 benign insulinomas (20). The present study describes new 68Ga- and 99mTc-labeled GLP-1 receptor agonists for PET/CT and SPECT/CT, respectively. The pharmacokinetics of the new compounds were compared with our gold standard [Lys40(Ahx-DOTA-111In)NH2]-exendin-4.

[Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 showed not only a fast, high, and specific uptake in the tumors but also a high tumor-to-background ratio. The high kidney uptake was significantly reduced by the administration of PGA, Gelofusine, or the combination of the 2 substances. In our mouse model, there was no significant difference in the biodistribution of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 and [Lys40(Ahx-DOTA-111In)NH2]-exendin-4. Accordingly, 111In- and 68Ga-labeled exendin-4 may show similar biodistributions and pharmacokinetics in humans. Two small tumors (1.5 and 2.3 mm) in the mouse pancreas were visualized using the same hybrid PET/CT camera as used for patients, showing the high potential of [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 PET in the detection of small tumors. PET has a higher sensitivity and spatial resolution than SPECT (29). This might be important because a high spatial resolution may facilitate the delineation of tumors and kidneys, especially at early time points after injection of the tracer. In a previous clinical study, we showed that the relatively low spatial resolution of [Lys40(Ahx-DOTA-111In)NH2]-exendin-4 SPECT is a relevant limitation of the method. In 2 of 6 patients, delineation of the tumor from the kidneys was possible only on late scans obtained more than 3 d after injection (20). In addition, a high sensitivity in tumor detection is desirable because 90% of insulinomas are small, with a diameter of less than 2 cm (30). Recent studies using 68Ga-labeled somatostatin receptor agonists showed a high sensitivity in the detection of somatostatin receptor subtype 2–expressing tumors (31–35).

68Ga is a highly suitable positron emitter for PET because it is a generator product with a half-life of 68 min that decays by 89% through positron emission (36). Importantly, the short half-life of 68Ga will cause lower radiation doses to patients than will [Lys40(Ahx-DOTA-111In)NH2]-exendin-4. In the RippiTag2 animal model, [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 showed rapid blood clearance and fast target localization, making short-lived [Lys40(Ahx-DOTA-68Ga)NH2]-exendin-4 a suitable PET tracer for GLP-1 insulinoma imaging.

Overexpression of GLP-1 receptors not only on insulinoma cells but also on pancreatic β-cells provides a further application of GLP-1 receptor imaging (37–39). Brom et al. evaluated noninvasive β-cell SPECT using the GLP-1 receptor agonist 111In-DTPA-exendin-3. They found a significant correlation between 111In-DTPA-exendin-3 uptake and β-cell mass in rats (40). GLP-1 receptor imaging is a noninvasive method with the potential to monitor the β-cell mass during the course of diabetes development and during antidiabetic treatment. Furthermore, the method might be used for noninvasive monitoring of islet cell graft survival after transplantation.

SPECT or SPECT/CT with 99mTc-labeled exendin-4 is an alternative approach to GLP-1 receptor imaging with 111In. The estimated effective dose of [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 is more than 40 times less than that of its 111In-labeled congener compound because of the low energy and short physical half-life of 99mTc and the significantly lower tumor and organ uptake of [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4, compared with [Lys40(Ahx-DOTA-111In)NH2]-exendin-4. These data can be explained by the significantly less efficient internalization. Regression analysis showed a significant correlation between the rate of internalization and the uptake in the tumor at 4 h after injection of the respective radiopeptide (Fig. 4). Despite lower tumor uptake, [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 multipinhole SPECT detected multiple small tumors (diameter, 1.0–3.2 mm) in the pancreas and is therefore a promising candidate for clinical GLP-1 receptor imaging studies.

In addition, 99mTc is a radionuclide suitable for detection with a γ-probe. Hence, intraoperative localization of insulinomas using a normal or endoscopic γ-probe might be an additional clinical application of [Lys40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4.

![FIGURE 4. Correlation of tumor uptake (%IA/g of tissue) and internalization (percentage of specific internalized/10⁶ cells) at 4 h. Each data point shows mean tumor uptake ± SD and mean internalization ± SD.](image-url)
CONCLUSION

These promising pharmacokinetic and imaging data show that [Lyso40(Ahx-DOTA-68Ga)NH2]-exendin-4 and [Lyso40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 are suitable candidates for clinical GLP-1 receptor imaging studies. PET/CT with [Lyso40(Ahx-DOTA-68Ga)NH2]-exendin-4 will possibly localize small insulinomas at early time points after injection, and SPECT/CT with [Lyso40(Ahx-HYNIC-99mTc/EDDA)NH2]-exendin-4 will potentially increase the availability of the method.

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