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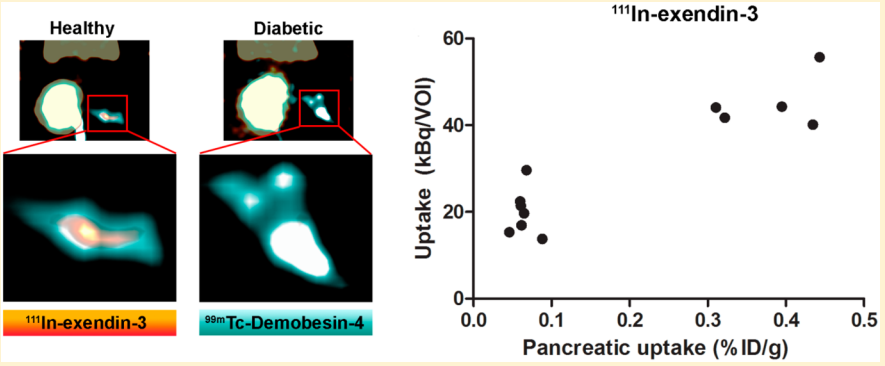
Improved Quantification of the Beta Cell Mass after Pancreas Visualization with ^{99m}Tc-demobesin-4 and Beta Cell Imaging with ^{111}In-exendin-3 in Rodents

Inge van der Kroon,*[,†] Lieke Joosten,[†] Berthold A. Nock,[‡] Theodosia Maina,[‡] Otto C. Boerman,[†] Maarten Brom,[†] and Martin Gotthardt[†]

[†]Department of Radiology and Nuclear Medicine, Radboud University Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands

[‡]Molecular Radiopharmacy, INRASTES, NCSR Demokritos, GR-153 10 Agia Paraskevi, Attikis, Athens, Greece

Supporting Information



ABSTRACT: **Objective:** Accurate assessment of the ^{111}In-exendin-3 uptake within the pancreas requires exact delineation of the pancreas, which is highly challenging by MRI and CT in rodents. In this study, the pancreatic tracer ^{99m}Tc-demobesin-4 was evaluated for accurate delineation of the pancreas to be able to accurately quantify ^{111}In-exendin-3 uptake within the pancreas. **Methods:** Healthy and alloxan-induced diabetic Brown Norway rats were injected with the pancreatic tracer ^{99m}Tc-demobesin-4 ([^{99m}Tc-N$_4$-Pro^1,Tyr^4,Nle^{14}]bombesin) and the beta cell tracer ^{111}In-exendin-3 ([^{111}In-DTPA-Lys^{40}]exendin-3). After dual isotope acquisition of SPECT images, ^{99m}Tc-demobesin-4 was used to define a volume of interest for the pancreas in SPECT images subsequently the ^{111}In-exendin-3 uptake within this region was quantified. Furthermore, biodistribution and autoradiography were performed in order to gain insight in the distribution of both tracers in the animals. **Results:** ^{99m}Tc-demobesin-4 showed high accumulation in the pancreas. The uptake was highly homogeneous throughout the pancreas, independent of diabetic status, as demonstrated by autoradiography, whereas ^{111}In-exendin-3 only accumulates in the islets of Langerhans. Quantification of both *ex vivo* and *in vivo* SPECT images resulted in an excellent linear correlation between the pancreatic uptake, determined with *ex vivo* counting and ^{111}In-exendin-3 uptake, determined from the quantitative analysis of the SPECT images (Pearson r = 0.97, Pearson r = 0.92). **Conclusion:** ^{99m}Tc-demobesin-4 shows high accumulation in the pancreas of rats. It is a suitable tracer for accurate delineation of the pancreas and can be conveniently used for simultaneous acquisition with ^{111}In labeled exendin-3. This method provides a straightforward, reliable, and objective method for preclinical beta cell mass (BCM) quantification with ^{111}In-exendin-3.

KEYWORDS: *exendin-3, demobesin-4, beta cell mass, pancreas imaging*

INTRODUCTION

One of the key pathophysiological processes involved in the development of diabetes mellitus is the loss of beta cells. An accurate method to quantify the beta cell mass (BCM) during this process is essential to gain more insight in the pathophysiology of diabetes and for the development of new therapeutic strategies. Currently, mainly autopsy data on BCM are available,[1,2] that only give information about the BCM at one time point in the course of the disease and simultaneous functional data seem to be missing in most cases. In the last couple of years, there have been several attempts to quantify the BCM noninvasively by imaging,[3] in order to provide real time information on the BCM during the development of diabetes.

A successful attempt to quantify the BCM was published by Brom et al.[4] They correlated radiolabeled exendin-3 uptake, as

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determined with SPECT imaging, with the histologically determined BCM in rats. With this method, exact delineation of the pancreas was challenging, especially in diabetic animals with low ^{111}In-exendin-3 uptake as a result of low BCM, in combination with the high renal uptake of ^{111}In-exendin-3 (>30% %ID/g), so that a certain volume of interest within the pancreas was used as a surrogate marker for total beta cell mass. Besides the beta cells, the duodenum and stomach, organs located closely to the pancreas potentially overlapping with the pancreas on SPECT, also express the glucagon-like peptide-1 receptor (GLP-1R)[5] and show accumulation of ^{111}In-exendin-3, which can lead to inaccurate estimations of the actual BCM. Unfortunately, it is not easily possible to use CT or MRI scans to delineate the pancreatic tissue in these animals because the pancreas of rodents cannot reliably be distinguished from the surrounding tissues on these scans.

To overcome this problem in SPECT imaging, a dual tracer imaging approach might be useful: ^{111}In-exendin-3 to quantify the BCM, and another tracer to accurately delineate the pancreatic tissue. In addition to the dual tracer imaging approach a unilateral nephrectomy was performed to enable quantification of the BCM of a larger part of the pancreas. After exact delineation of the pancreas, the ^{111}In-exendin-3 uptake within this region can be quantified. The use of two radionuclides for labeling, with different energy peaks, enables simultaneous acquisition of both images. Such a method would simplify the BCM quantification procedure and would be less vulnerable to interindividual assessment differences due to manually drawn ROIs around the pancreas.

A potential tracer to accurately visualize the exocrine part of the pancreas is ^{99m}Tc-demobesin-4. ^{99m}Tc-Demobesin-4 binds to the gastrin releasing protein receptor (GRPR) physiologically expressed in the pancreas and is mainly used for visualization of GRPR positive tumors.[6−8] Besides this high uptake in GRPR positive tumors, it was also shown that several other bombesin analogs have high uptake in the exocrine part of the pancreas.[6,9] The GRPR agonists, like ^{99m}Tc-demobesin-4 show higher pancreatic retention than the previously tested antagonist ^{99m}Tc-demobesin-1.[6,9] ^{99m}Tc-demobesin-4 therefore has the potential to improve the visualization of the pancreas and thereby aid the quantification of ^{111}In-exendin-3 signal within the pancreas.

In this study, the feasibility to accurately determine the BCM by SPECT imaging in Brown Norway rats after coinjection of ^{99m}Tc-demobesin-4 and ^{111}In-exendin-3 was evaluated. To investigate the possibility to reliably detect even small amounts of beta cells one group of animals received alloxan to reduce the BCM in the animals.

MATERIALS AND METHODS

Peptides and Radiolabeling. *^{111}In-exendin-3.* [Lys^{40}]-(DTPA)]exendin-3 (200 pmol) (Peptides Specialty Laboratories, Heidelberg, Germany) (referred to as exendin-3 in the remainder of the text) was incubated with $^{111}InCl_3$ (150 MBq) (Mallinckrodt Pharmaceuticals, 's-Hertogenbosch, The Netherlands) in 0.5 M MES buffer (Sigma-Aldrich, St Louis, MO, U.S.A.), pH 5.5 at room temperature. After incubation for 20 min, 50 mM EDTA (Sigma-Aldrich, St Louis, MO, U.S.A.) was added to reach a final concentration of 5 mM and 10% Tween80 (Sigma-Aldrich, St Louis, MO, U.S.A.) to a final concentration of 0.1%. The labeling efficiency was determined on silica gel instant thin layer chromatography (ITLC) using 0.1 M EDTA in 0.1 M NH_4Ac pH 5.5 (R_f = 0, ^{111}In-exendin-3; R_f = 1, ^{111}In-EDTA). The reaction mixture was purified by solid-phase extraction using an HLB reversed-phase sorbent cartridge (30 mg, Water Oasis, Milford, MA, U.S.A.), as previously described.[10] After purification the radiochemical purity was >99%, with an average radiochemical yield of ∼60%

^{99m}Tc-demobesin-4. Demobesin-4 was provided by the Molecular Radiopharmacy, NCSR Demokritos (Athens, Greece) and dissolved in 0.1% acetic acid in water and ethanol (8:2, v/v) at a final concentration of 1.8 mg/mL; aliquots thereof were stored at −20 °C. Demobesin-4 (2.8 nmol) was labeled with ^{99m}Tc pertechnetate (83 μL, 100−120 MBq) in 10 μL of 0.5 M phosphate buffer (pH 11.5), 1 μL of 0.1 M sodium citrate and 3 μL of freshly prepared $SnCl_2$ (Sigma-Aldrich) in pure ethanol (2 mg/mL). The mixture was incubated at room temperature for 30 min. After incubation, the pH of the solution was reduced to 7 by addition of 1 M HCl (4 μL), and 10% Tween80 was added to a final concentration of 0.1%. Radiochemical purity was determined by ITLC, using two mobile phases: 100% aceton (R_f = 0, ^{99m}Tc-demobesin-4, hydrolyzed ^{99m}Tc; R_f = 1, $^{99m}TcO_4^-$) and 100% MeOH in 0.1 M NH_4Ac (1:1) (R_f = 0, hydrolyzed ^{99m}Tc; R_f = 1, ^{99m}Tc-demobesin-4, ^{99m}Tc-citrate, $^{99m}TcO_4^-$) or reversed-phase high performance liquid chromatography (RP-HPLC) on a C-18 Alltech Alltima column, 4.6 × 250 mm, 5 μm (Fisher Scientific, U.S.A.). The column was eluted with 0.1% TFA in H_2O with a linear gradient from 10 to 40% acetonitrile in 30 min followed by a linear gradient from 40% to 100% in 10 min (flow rate 1 mL/min). Under these conditions ^{99m}Tc-citrate elutes at 2.5 min, $^{99m}TcO_4^-$ at 3.4 min, and ^{99m}Tc-demobesin-4 at 29.1 min. The radiochemical purity of ^{99m}Tc-demobesin-4 always exceeded 98%, and because no purification was involved, a similar radiochemical yield was reached.

Animal Experiments. Animal experiments were performed in six to eight week old Brown Norway rats (150 ± 7g), purchased from Charles River (Sulzfeldt, Germany). The animal experiments were approved by the animal welfare committee of the Radboud University Nijmegen. A unilateral nephrectomy of the left kidney was performed under inhalation anesthesia with isoflurane in O_2 and air (induction 4−5%, maintenance 1.5−2%) to improve visualization of the pancreas for quantification purposes. Prior to isoflurane anesthetics rats were given Carprofen (5 mg/kg s.c., Rimadyl, Pfizer Animal Health B.V., Netherlands) for analgesia. Carprofen injections were repeated twice daily for 2 days after surgery.

Alloxan Treatment. One week after surgery the rats were injected with 45 or 60 mg/kg body weight alloxan monohydrate (Sigma Chemical, St. Louis, MO, U.S.A.) (n = 4/group). Alloxan was dissolved in PBS (0.1 mg/μL), and protected from light and kept on ice before intravenous (i.v.) injection. Control rats were injected with PBS only.

SPECT/CT Acquisition. One week after alloxan treatment blood glucose concentrations were measured and when hyperglycemia was confirmed in treated rats, diabetic (blood glucose: 32 ± 5.9 mmol/L) and control rats (blood glucose 6.5 ± 0.7 mmol/L) were i.v. injected with 14 ± 1.2 MBq ^{111}In-exendin-3 (peptide dose 20 pmol, 200 μL) and 17 ± 2.8 MBq ^{99m}Tc-demobesin-4 (peptide dose 0.56 nmol, 200 μL). Six healthy rats received a coinjection with either an excess of unlabeled exendin-3 (5 nmol) or an excess of unlabeled demobesin-4 (56 nmol) (n = 3/group) to determine whether the uptake of the tracers is GLP-1R or GRPR mediated.

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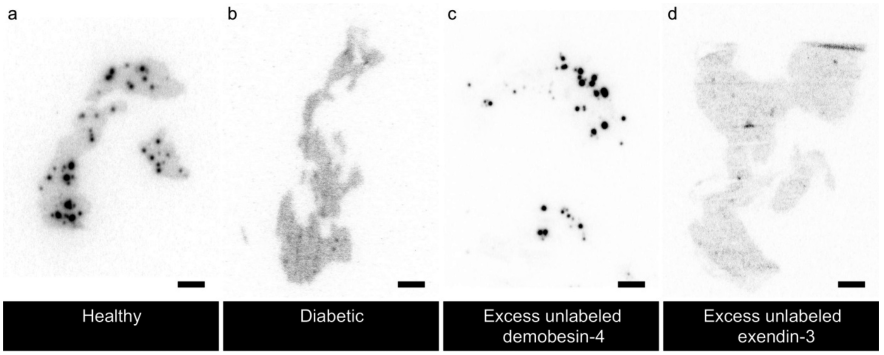

Figure 1. Digital autoradiography of pancreatic sections (4 μm) of healthy (a), (c), (d) and alloxan-treated (60 mg/kg) (b) rats. All rats were injected with 14 ± 1.2 MBq ^{111}In-exendin-3 and 17 ± 2.8 MBq ^{99m}Tc-demobesin-4. In addition the rat in (c) received an excess of unlabeled demobesin-4, whereas the rat in (d) received an excess of unlabeled exendin-3. In (a),(b),(d) a homogeneous accumulation of ^{99m}Tc-demobesin-4 was observed in the exocrine pancreas; this uptake could be blocked by an excess of unlabeled demobesin-4, although accumulation of the ^{111}In-exendin-3 in the islets was still present (c). Injection of an excess of unlabeled exendin-3 blocked the uptake of ^{111}In-exendin-3 in the islets, whereas the homogeneous uptake of ^{99m}Tc-demobesin-4 was still present (d). The black bars represent 2 mm.

One hour after injection of the radiolabeled compounds SPECT/CT images were acquired (U-SPECT-II, MILabs, Utrecht, The Netherlands). Rats were scanned under general anesthesia (isoflurane in air) for 50 min using a 1.0 mm multipurpose rat and mouse collimator.

Biodistribution. After the imaging procedure, rats were euthanized by CO_2/O_2 suffocation. Tissues were dissected, weighed and the uptake of the radiotracers in the tissues was determined using a gamma counter (Wallac 1480 wizard, PerkinElmer, Waltham, MA, U.S.A.) with reference solutions of ^{99m}Tc and ^{111}In (200 μL samples containing 1% and 0.1% of the injected dose).

Counting was performed on the 140.5 keV for ^{99m}Tc (120−140 keV) and the 171 and 245 keV photopeaks for ^{111}In (150−541 keV). The counting windows were adjusted to minimize contamination of ^{111}In activity in the ^{99m}Tc window. Only when the amount of ^{111}In in a tissue was several times higher than the amount of ^{99m}Tc, the counts in the ^{99m}Tc window were significantly contaminated with counts from the ^{111}In-window.

The uptake of ^{111}In-exendin-3 in the tissues was measured after decay of the ^{99m}Tc. Uptake in the tissues was expressed as percentage of injected activity per gram of tissue (%ID/g). Immediately after dissection, all pancreata were transferred to 1.5 mL Eppendorf tubes containing 4% formalin, and subsequent embedding in paraffin.

***Ex Vivo* SPECT Acquisition.** Pancreata were dissected and each pancreas was transferred to a 1.5 mL Eppendorf tube containing 4% formalin immediately after dissection. For each acquisition three or four pancreata (in total 11 pancreata (3 control, 4 treated with 45 mg/kg alloxan, 4 treated with 60 mg/kg alloxan)) in Eppendorf tubes were placed in a mouse animal bed and subsequently *ex vivo* SPECT images were acquired, using a 1 mm ultrahigh sensitivity mouse collimator (MILabs, Utrecht, The Netherlands) with an acquisition time of 6 h.

SPECT/CT Reconstruction and Quantification. SPECT images were reconstructed using the U-SPECT-Rec software (MILabs, Utrecht, The Netherlands). The ^{111}In and ^{99m}Tc images were reconstructed separately. For ^{111}In-exendin-3 images, only counts from the 245 keV photopeak (223−259 keV) of ^{111}In were reconstructed. Since no counts from ^{99m}Tc were measured in this window the quantification of the ^{111}In images was only based on uptake of ^{111}In in the beta cells. ^{99m}Tc-demobesin-4 images were reconstructed with counts from the 140.5 keV photopeak of ^{99m}Tc. In this window, there are also counts from the 171 keV photopeak of ^{111}In present, but the ^{99m}Tc images were only used for delineation of the pancreatic tissue and not for quantification. The contribution of the counts from the 171 keV ^{111}In photopeak in the ^{99m}Tc window depends on the biodistribution of both tracers.

Images were analyzed using Inveon Research Workplace (Siemens Healthcare, Den Haag, The Netherlands). The ^{111}In-exendin-3 images were used to draw a volume of interest (VOI) around the kidney, this VOI was dilated with a radius of 1 mm. Subsequently, the kidney VOI was copied to the ^{99m}Tc-demobesin-4 image. The uptake in the kidney VOI was excluded. The pancreas was selected by drawing a cubical VOI around the pancreas in the ^{99m}Tc-demobesin-4 window and subsequently the 50% hottest pixels within this VOI were selected.

This VOI of the pancreas in the ^{99m}Tc-demobesin-4 image was copied to the ^{111}In-exendin-3 image and the signal inside the VOI was measured. Standards with a known amount of ^{111}In (0, 18.5, 37, 55.5 kBq) were scanned, quantified, and used to determine a calibration factor to convert the SPECT signal to uptake in kBq.

***Ex vivo* Autoradiography.** *Ex vivo* autoradiography was performed to visualize the distribution of the tracers in the pancreas. Sections (4 μm) were exposed to a phosphor imaging plate (Fuji Film BAS-SR 2025, Raytest, Straubenhardt, Germany) for 24 h at room temperature, to visualize uptake of ^{99m}Tc-demobesin-4 and ^{111}In-exendin-3. After 24 h, the imaging plate was developed using a radioluminography laser imager (Fuji Film BAS 1800 II system, Raytest, Straubenhardt, Germany). Subsequently, the same tissue sections were exposed for 6 days to a phosphor imaging plate to visualize only uptake of ^{111}In-exendin-3. Images were analyzed with Aida image Analyzer software (Raytest, Straubenhardt, Germany).

Statistical Analysis. All mean values are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using Graphpad Prism (version 5.0.3). Correlations were determined using the Pearson correlation coefficient (r). The level of significance was set at $p < 0.05$.

RESULTS

***Ex Vivo* Autoradiography.** Autoradiography of pancreatic sections of healthy and alloxan treated rats showed high tracer accumulation of ^{111}In-exendin-3 restricted to the islets of Langerhans and a homogeneous uptake of ^{99m}Tc-demobesin-4 within the pancreas (Figure 1). Uptake of both tracers was

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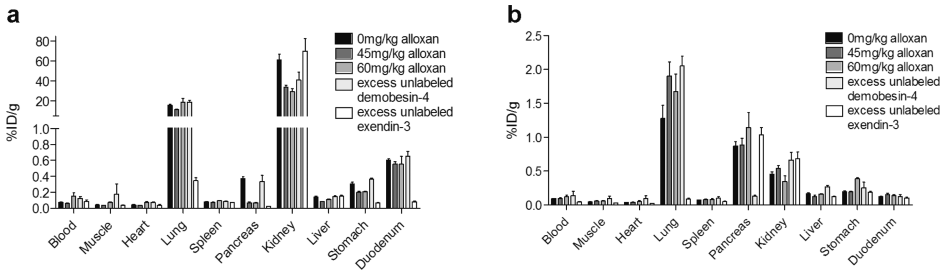

Figure 2. Biodistribution data of (a) ^{111}In-exendin-3 and (b) ^{99m}Tc-demobesin-4 in control and alloxan treated rats. Blocking was performed by coinjection of an excess of unlabeled exendin-3 or an excess of unlabeled demobesin-4.

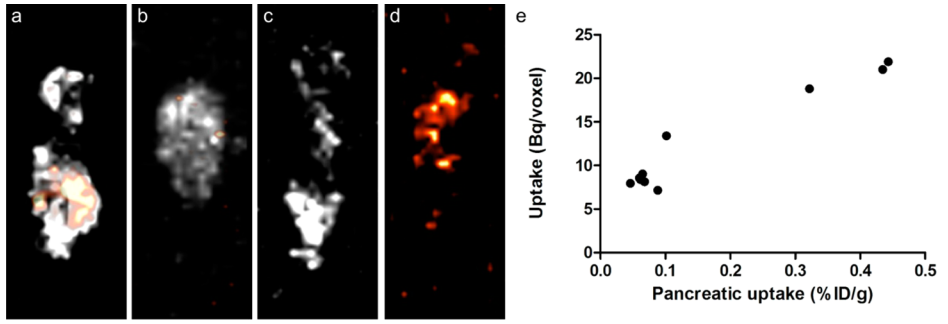

Figure 3. *Ex vivo* SPECT images of a healthy (a), an alloxan treated pancreas (60 mg/kg) (b) and a rat coinjected with either an excess of unlabeled demobesin-4 or exendin-3 (c and d) (^{99m}Tc-demobesin-4 is shown in gray, ^{111}In-exendin-3 is shown in yellow). Correlation between uptake of ^{111}In-exendin-3 determined by SPECT and biodistribution data is shown in (e), with a Pearson correlation coefficient of $r = 0.97$, $p < 0.0001$. The uptake on the y axis is expressed in Bq/voxel, and the uptake determined with the gamma counter is expressed as %ID/g.

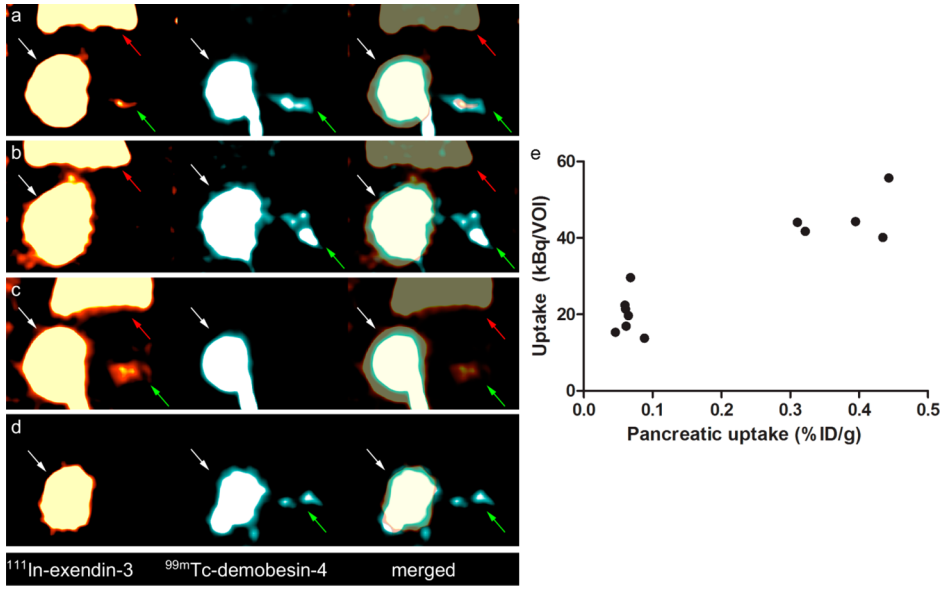

Figure 4. *In vivo* SPECT images of a healthy (a), an alloxan treated rat (60 mg/kg) (b) and rats coinjected with an excess of unlabeled demobesin-4 and exendin-3, respectively (c and d). ^{111}In-exendin-3 uptake is shown in orange/yellow and ^{99m}Tc-demobesin-4 uptake is shown in blue/white. Green arrow, pancreas; white arrow, kidney; red arrow, lungs. Correlation between the uptake of ^{111}In-exendin-3 determined by SPECT and the biodistribution data are shown in (e) (Pearson $r = 0.92$, $p < 0.0001$). The uptake on the y axis is expressed in kBq/VOI, whereas the uptake on the x axis is expressed as %ID/g.

receptor mediated because it could be blocked by an excess of unlabeled tracer (exendin-3 or demobesin-4) (Figure 1c,d).

Biodistribution. ^{111}In-exendin-3 showed a biodistribution pattern as previously described,[4] with high specific uptake in the lungs (>10%ID/g) and high nonreceptor mediated accumulation in the kidneys (>40%ID/g). Furthermore, specific uptake was observed in the pancreas of healthy rats and stomach and duodenum of both healthy and alloxan-treated rats because coinjection of an excess of unlabeled exendin-3 blocked uptake of the ^{111}In-exendin-3 (Figure 2a).

The concentration of ^{99m}Tc-demobesin-4 in the pancreas was 0.98 ± 0.27%ID/g, which could be blocked by coinjection of an excess of unlabeled demobesin-4 (0.13 ± 0.03%ID/g), indicating GRPR mediated uptake. The uptake in all other organs was low

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(<0.5%ID/g), except in the lungs. The uptake of ^{99m}Tc-demobesin-4 in the lungs was overestimated due to contamination of the ^{99m}Tc-window with ^{111}In. This contamination is a result of the high uptake of ^{111}In-exendin-3 in the lungs (confirmed in the excess exendin-3 group, where the ^{111}In-exendin-3 uptake in the lungs was blocked (Figure 2b). Unfortunately, it was not possible to accurately determine the ^{99m}Tc activity in the kidneys, due to the extremely high kidney uptake of ^{111}In-exendin-3. This leads to an underestimation of the %ID/g ^{99m}Tc in the kidneys. On the basis of biodistribution data of animals injected with only ^{99m}Tc, a kidney uptake of approximately 2.5%ID/g is expected (data not shown).

***Ex Vivo* SPECT of the Pancreas.** The uptake of ^{99m}Tc-demobesin-4 was clearly visualized in the pancreas of healthy and alloxan treated animals (Figure 3), whereas coinjection of an excess of unlabeled demobesin-4 blocked the receptor mediated pancreatic uptake of ^{99m}Tc-demobesin-4. ^{111}In-exendin-3 uptake colocalized with ^{99m}Tc-demobesin-4 uptake, where hardly any ^{111}In-exendin-3 uptake was observed in alloxan treated rats. Calculation of the correlation coefficient between the biodistribution data and ^{111}In-exendin-3 uptake as determined by SPECT revealed a correlation coefficient (Pearson r) of 0.97 ($p < 0.0001$) (Figure 3e).

Quantitative Analysis of *In Vivo* SPECT Images. Specific accumulation of ^{99m}Tc-demobesin-4 was observed in pancreatic tissue. In healthy animals, ^{111}In-exendin-3 uptake in the pancreas colocalized with the ^{99m}Tc-demobesin-4 uptake. In diabetic animals, negligible uptake of ^{111}In-exendin-3 was observed within the VOI of the pancreas, based on ^{99m}Tc-demobesin-4 uptake (Figure 4a,b). Quantification of the ^{111}In-exendin-3 uptake within the VOI based on the ^{99m}Tc-demobesin-4 images linearly correlated with the pancreatic uptake based on biodistribution data (Figure 4e, Pearson $r = 0.92$). Coinjection of an excess of unlabeled peptide-ligand, either exendin-3 or demobesin-4, blocked the specific tracer accumulation (Figure 4c,d).

DISCUSSION

Radiolabeled exendin-3 is a promising tracer for BCM imaging in both the native pancreas[4,11−15] and after islet transplantation.[16,17] However, exact delineation of the native pancreatic tissue is difficult and time-consuming. Brom et al. demonstrated the potential of radiolabeled exendin-3 not only for qualitative imaging of the BCM but also for exact quantification of the BCM. In this study, a linear correlation between BCM and ^{111}In-exendin-3 uptake was observed, with a Pearson correlation coefficient of 0.89.[4] The quantification was based on the uptake in a small VOI in the pancreas, just above the kidney, which was used as a surrogate marker for total pancreatic BCM. In addition to the difficult delineation of the pancreas, the high kidney uptake of ^{111}In-exendin-3 (>30% ID/g in rats) also hampers visualization of the ^{111}In-exendin-3 uptake in the pancreas in rodents.

Because the pancreas is not clearly distinguishable from surrounding tissue on CT images in rodents and reduction of the high kidney uptake is difficult to achieve,[18−20] quantification of the BCM in a larger VOI was not possible. To overcome these problems, one kidney was removed in our experiments and an additional tracer to visualize the whole pancreas was used. An additional tracer not only facilitates accurate delineation of pancreatic tissue and provides a straightforward, reliable, and objective method for BCM quantification, but it also allows quantification of the BCM in a larger VOI. The use of an exocrine tracer will not be necessary in a clinical setting because the pancreas morphology in humans is more defined than in rodents and therefore better visible on CT images. CT images could be used to delineate the pancreatic tissue and quantify the uptake of the BCM marker within this volume of interest.

In this study, the potential of ^{99m}Tc-demobesin-4 as tracer for the exocrine pancreas, allowing exact delineation of the pancreas in beta cell imaging, was evaluated in a rat model. This tracer shows not only high uptake in rats with a high BCM, but also in rats with low BCM due to the expression of GRPR in the exocrine pancreas which is not influenced by loss of beta cell mass. As rats represent the preferred model for BCM imaging in vivo,[21] quantification of the BCM in rats with low BCM is warranted in preclinical diabetes research but also is challenging, and therefore, the need for an exocrine pancreas tracer is clear. Previously, Mikkola et al. used ^{11}C-methionine to verify the location of the pancreas, but in this study, the pancreatic tracer was not used for quantification purposes of a beta cell marker.[11] More recently, Mathijs et al. used the iodinated amino acid ^{123}Iodo-L-phenylalanine[15] to determine the pancreatic VOI for quantification of ^{111}In-exendin-3 in a dual tracer imaging approach. They showed a Pearson correlation coefficient of $r = 0.83$ between pancreatic uptake as determined in the gamma counter and SPECT quantification.[15] In our study, we were able to achieve an excellent correlation between ^{111}In-exendin-3 uptake quantified on *in vivo* SPECT images and *ex vivo* counting (Pearson $r = 0.92$, $p < 0.0001$). *Ex vivo* scanning of the pancreas and quantification resulted in an almost perfect correlation with *ex vivo* counting (Pearson $r = 0.97$). These results clearly demonstrate that ^{99m}Tc-demobesin-4 in combination with surgical removal of the left kidney is indeed highly suitable for delineation of the pancreatic region for BCM quantification purposes. Besides this excellent correlation between the quantified *in vivo* ^{111}In-exendin-3 uptake in SPECT images and *ex vivo* counting, ^{99m}Tc-demobesin-4 offers two additional advantages over the ^{123}I-labeled exocrine tracer. Deiodination of the ^{123}Iodo-L-phenylalanine in vivo and consequent radioiodine release might increase radioactivity levels in the stomach, an organ close to the pancreas, and thereby hinder exact delineation of the pancreas. Another complicating factor for quantification purposes of the pancreas with ^{123}Iodo-L-phenylalanine is the close proximity of the ^{123}I peak to the low energy peak of ^{111}In. ^{111}In can contaminate the ^{123}I window, and the high uptake of ^{111}In in the stomach duodenum transition might be seen as ^{123}I uptake.

In addition to using an exocrine pancreas tracer to improve BCM quantification, a unilateral nephrectomy (left kidney) was performed, to enable quantification of the BCM in a larger area of the pancreas. This is of importance because islets are heterogeneously distributed throughout the pancreas and therefore quantification of the uptake in a small VOI could lead to less accurate BCM estimations. The unilateral nephrectomy did not influence the biodistribution pattern of either ^{111}In-exendin-3 or ^{99m}Tc-demobesin-4 (Supporting Information). Both tracers are cleared via the kidneys, and because of the nephrectomy, all activity is cleared via one kidney, resulting in a higher radiation burden. Consequently, multiple injections might finally induce radiation damage to the kidneys, thereby potentially influencing the biodistribution of the tracer. Although this model might not prove optimal in longitudinal studies, the nephrectomy involved is necessary for accurate validation of the tracer in a preclinical setting. This advantage clearly outweighs the drawbacks.

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In conclusion, the exocrine pancreatic marker ^{99m}Tc-demobesin-4 showed high uptake in the pancreas, not only in animals with high BCM but also in animals with low BCM. The combination of using ^{99m}Tc-demobesin-4 as an exocrine tracer (for delineation of the complete pancreas) and performing a unilateral nephrectomy allowed to precisely quantify the uptake in the total pancreas, resulting in a more robust determination of the uptake of ^{111}In-exendin-3 in the pancreas of rodents. Therefore, we clearly suggest to use this model in future rodent studies with radiolabeled exendin-3 as a straightforward and objective quantification method.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.6b00495.

Biodistribution data of both ^{111}In-exendin-3 and ^{99m}Tc-demobesin-4 in rats without nephrectomy. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +31 24 36 19097. Fax: + 31 24 36 18942. E-mail: Inge.vanderKroon@radboudumc.nl.

Notes

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