

In Vitro and In Vivo Characterization of Three ^{68}Ga - and ^{111}In -Labeled Peptides for Cholecystokinin Receptor Imaging

Susan Roosenburg, Peter Laverman, Lieke Joosten, Annemarie Eek, Floris P.J.T. Rutjes, Floris L. van Delft, and Otto C. Boerman

Abstract

Cholecystokinin (CCK) receptors are overexpressed in several human tumor types, such as medullary thyroid carcinomas and small cell lung cancers. Several ligands for the CCK2 receptor (CCK2R) have been developed for radionuclide targeting of these tumors. In this study, we evaluated whether radiolabeled DOTA-sCCK8 and its stabilized derivative, DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}], are suitable for imaging of CCK2R-positive tumors, using DOTA-MG0 as a reference. In vivo targeting of CCK2R-positive tumors with DOTA-sCCK8, DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}], and DOTA-MG0, labeled with ^{111}In or ^{68}Ga , was evaluated in BALB/c nude mice with a subcutaneous A431-CCK2R tumor. Biodistribution studies and single-photon emission computed tomography (SPECT) and positron emission tomography (PET) were performed at 1 hour postinjection. All peptides specifically accreted in the CCK2R-expressing tumors. Both ^{111}In -DOTA-sCCK8 and ^{111}In -DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] showed good tumor retention (4.65% ID/g and 5.44% ID/g, respectively, at 4 hours postinjection). On PET/computed tomographic (CT) and SPECT/CT scans, subcutaneous A431-CCK2R tumors were clearly visualized with low uptake of sCCK8 peptides in the intestines. Whereas radiolabeled DOTA-MG0 showed high kidney uptake (70% ID/g), the sCCK8 peptides showed low uptake in the kidneys. Sulfated CCK8 analogues combined high tumor uptake with low retention in the kidney and are therefore promising tracers for imaging of CCK2R-positive tumors.

THE DEVELOPMENT OF RADIOPHARMACEUTICALS for diagnostic and therapeutic purposes based on regulatory peptides is of particular interest in oncology as many receptors are overexpressed in different types of human cancers, for example, somatostatin receptors, gastrin-releasing peptide receptors, and gastrin receptors. In this study, we focused on the cholecystokinin receptor (CCKR) as a target for peptide receptor radionuclide imaging and therapy. The CCK2 receptor (CCK2R) is abundantly expressed in most medullary thyroid carcinomas and small cell lung cancers, as well as in stromal ovarian cancers and astrocytomas.¹

In earlier studies, a gastrin analogue (minigastrin = MG0) was explored as a therapeutic agent for radionuclide targeting of CCK2R-positive tumors. MG0 is a peptide

that binds selectively to the CCK2R and has a high tumor uptake in CCK2R-positive tumors. However, MG0 also displays high kidney uptake, which may hamper visualization of tumors in the vicinity of the kidneys but is especially a problem when using this peptide for therapeutic applications.^{1,2} As an alternative to MG0, sCCK8 peptides display affinity for both the CCK1 receptor (CCK1R) and the CCK2R, whereas uptake in the kidneys is relatively low. A disadvantage of sCCK8 compared to MG0 is that sCCK8 contains an easily hydrolyzable sulfated tyrosine (Tyr) residue. Apart from that, the sCCK8 sequence contains two methionines (Met) that are prone to oxidation during radiolabeling and potentially in vivo.^{3,4} In our previous studies, we showed that the stability of sCCK8 can be increased by replacing the Tyr sulfate moiety by phenylalanine sulfonate (Phe(*p*-CH₂SO₃H)) and that the Met residues can be replaced by norleucine (Nle) with minimal impact on the receptor affinity.⁵

In this study, we compared the in vitro and in vivo properties of DOTA-sCCK8, DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}], and DOTA-MG0. The peptides were labeled with either ^{111}In or ^{68}Ga and evaluated in terms of

From the Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, and Institute for Molecules and Materials, Radboud University Nijmegen, Nijmegen, the Netherlands.

Address reprint requests to: Peter Laverman, PhD, PO Box 9101, 6500 HB, Nijmegen, the Netherlands; e-mail: p.laverman@nucmed.umcn.nl.

DOI 10.2310/7290.2012.00001

© 2012 Decker Publishing

DECKER

50% inhibitory concentration (IC_{50}) and biodistribution. Targeting of CCK2R-positive tumors with the radiolabeled peptides was visualized by micro-single-photon emission computed tomography (SPECT)/computed tomography (CT) (^{111}In -labeled peptides) and micro-positron emission tomography (PET)/CT (^{68}Ga -labeled peptides).

Materials and Methods

Peptides and Radionuclides

DOTA-sCCK8 was obtained from piChem, GmbH (Graz, Austria). DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] was synthesized as described previously.⁵ DOTA-MG0 was obtained from Peptide Specialty Laboratories (PSL GmbH, Heidelberg, Germany). The amino acid sequences of the peptides are depicted in Table 1.

$^{111}\text{InCl}_3$ was obtained from Covidien (Petten, the Netherlands). $^{68}\text{GaCl}_3$ was eluted from a TiO₂-based 1,850 MBq $^{68}\text{Ge}/^{68}\text{Ga}$ generator (IGG-100, Eckert & Ziegler, Berlin, Germany) with 6 mL of 0.1 N Ultrapure HCl (J.T. Baker, Deventer, the Netherlands). The $^{68}\text{GaCl}_3$ was collected in four fractions of 1.5 mL; the fraction containing the majority of ^{68}Ga activity was used for radiolabeling.

Radiolabeling

The DOTA-conjugated peptides were radiolabeled with ^{111}In in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 5.5, for 20 minutes at 95°C. After incubation, 10 mM ethylenediaminetetraacetic acid (EDTA) was added to a final concentration of 1 mM. Labeling efficiency and radiochemical purity were checked by high-performance liquid chromatography (1100 series LC system, Agilent Technologies, Palo Alto, CA; Alltima RP-C18 column 5 μm , 4.6 \times 250 mm, Alltech, Deerfield, IL) with 0.1% trifluoroacetic acid with a gradient of 5 to 95% MeCN at a flow rate of 1 mL/min. Instant thin-layer chromatography (ITLC) was performed on ITLC-silica gel strips (Agilent Technologies) with mobile phase A (0.1 M NH₄OAc/0.1 M EDTA [1/1 v/v]

[R_F labeled peptide = 0, R_F unbound ^{111}In = 1]) and mobile phase B (MeOH/3.5% NH₃ [1/1 v/v] [R_F colloid = 0, R_F unbound ^{111}In + labeled peptide = 1]) as eluents. The radiochemical purities of the peptides used in the studies described here were always above 95%.

Radiolabeling of the peptides with ^{68}Ga was performed in 2.5 M 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer (Sigma Chemicals, St. Louis, MO). $^{68}\text{GaCl}_3$ (37 MBq) was added to 120 μL HEPES buffer (2.5 M, pH 5.6) containing 1 μg (0.47–0.67 nmol) DOTA peptide. The final pH of the labeling mixture was 3.5. After incubation at 95°C for 15 minutes, 10 mM EDTA was added to a final concentration of 1 mM. The reaction mixture was purified on a C-18 cartridge (hydrophilic-lipophilic balance [HLB]; Waters, Inc, Milford, MA) and eluted with ethanol.

Cell Culture

In these studies, A431 cells transfected with CCK2R and A431 cells transfected with a control transfectant were used. Cells were constructed as described previously.⁶ Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 4.5 g/L D-glucose (Gibco, Invitrogen, Breda, the Netherlands) supplemented with 10% fetal calf serum and 250 $\mu\text{g}/\text{mL}$ G418 (Geneticin) in a humidified 5% CO₂ atmosphere at 37°C. The cells were harvested by trypsinization with trypsin/EDTA.

IC₅₀ Determination

The apparent IC_{50} of the peptides labeled with ^{115}In or ^{69}Ga for binding the CCK2R was determined on A431-CCK2R cells. Cells were grown to confluency in six-well plates. Cells were washed with binding buffer (DMEM supplemented with 0.5% w/v bovine serum albumin [BSA]) and incubated at room temperature for 10 minutes in binding buffer. Subsequently, peptide labeled with ^{115}In or ^{69}Ga was added at final concentrations ranging from 0.1 to 1,000 nM. Then a trace amount of ^{111}In -DOTA-sCCK8 (40,000 cpm/well) was added. After incubation at room temperature for 2 hours, binding buffer was removed, cells

Table 1. Amino Acid Sequence of DOTA Peptides

Peptide	Amino Acid Sequence	Molecular Weight (Da)
DOTA-sCCK8	DOTA-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-Asp-Phe-NH ₂	1,529.7
DOTA-sCCK8[Phe ² (<i>p</i> -CH ₂ SO ₃ H), Nle ^{3,6}]	DOTA-D-Asp-Phe(<i>p</i> -CH ₂ SO ₃ H)-Nle-Gly-Trp-Nle-Asp-Phe-NH ₂	1,490.2
DOTA-minigastrin (DOTA-MG0)	DOTA-D-Glu-(Glu) ₅ -Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH ₂	2,112.5

were washed twice with binding buffer, and cell-associated radioactivity was determined. The apparent IC_{50} was defined as the peptide concentration at which 50% of binding without competitor was reached. IC_{50} values were calculated using *GraphPad Prism* software version 4.00 for Windows (GraphPad Software, San Diego, CA).

Biodistribution Studies of ^{111}In -Labeled Peptides

Tumor targeting of ^{111}In -labeled DOTA peptides was investigated in female athymic BALB/c nude mice. Subcutaneous tumors were induced by inoculation with CCK2R-expressing A431 cells. Mice were inoculated with 2×10^6 A431-CCK2R cells in the right flank and 2×10^6 A431-mock cells in the left flank. When tumors had reached a weight of approximately 0.2 g (as measured by caliper measurements), mice were divided into groups ($n = 5/\text{group}$), and 185 kBq of ^{111}In -DOTA-peptide (0.05 μg , 0.03 nmol) was injected intravenously. Specificity was studied in groups that received a 1,000-fold molar excess of unlabeled peptide. Groups of mice were killed 1 hour and 4 hours postinjection, a blood sample was drawn, and organs of interest were dissected, weighed, and counted in a gamma-counter along with a standard of the injected activity to allow calculation of the injected dose per gram of tissue (%ID/g). Animal experiments were approved by the local animal welfare committee and carried out according to national regulations.

Biodistribution Studies of ^{68}Ga -Labeled Peptides

Tumor targeting of ^{68}Ga -labeled DOTA peptides was investigated as described above for the ^{111}In -labeled peptides. In this study, 2 MBq of ^{68}Ga -DOTA-peptide (0.05 μg , 0.03 nmol) was injected intravenously after the tumors had reached a weight of 0.2 g. Mice were killed 1 hour postinjection.

SPECT/CT Imaging Studies

BALB/c nude mice with a subcutaneous A431-CCK2R tumor (diameter 2–5 mm) on one shoulder and an A431-mock tumor on the contralateral shoulder received an intravenous injection of 4.1 to 4.8 MBq of ^{111}In -labeled peptide (specific activity 67–87 MBq/nmol). At 1 and 4 hours SPECT images were acquired with a U-SPECT-II/CT scanner (MILabs, Utrecht, the Netherlands). Mice were scanned in the prone position under general anesthesia (isoflurane and O_2) for 30 to 60 minutes using a 1.0 mm diameter pinhole rat collimator tube, followed by a CT

scan (spatial resolution 160 μm , 40 kV, 612 μA) for anatomic reference. At 4 hours postinjection, the mice were euthanized and scanned, and after the scan was recorded, the uptake of ^{111}In -labeled peptide in dissected tissue was determined as described for the biodistribution study. Scans were reconstructed with software from MILabs, which uses an ordered-subset expectation maximization algorithm, with a voxel size of 0.375 mm. Scans were analyzed *Inveon Research Workplace* software version 3.0 (Preclinical Solutions, Siemens Healthcare Molecular Imaging, Knoxville, TN).

PET/CT Imaging Studies

PET images were acquired of BALB/c nude mice with a subcutaneous A431-CCK2R tumor on one shoulder and an A431-mock tumor on the contralateral shoulder using a small-animal PET-CT scanner (Inveon, Preclinical Solutions, Siemens Healthcare Molecular Imaging). When the tumors had a diameter of 2 to 5 mm, the mice were injected intravenously with 4.2 to 5.4 MBq of ^{68}Ga -DOTA peptide (50–58 MBq/nmol). Mice were euthanized by CO_2/O_2 suffocation and were scanned in the prone position. PET images were acquired for 30 minutes, followed by a CT scan (spatial resolution 113 μm , 80 kV, 500 μA). Scans were reconstructed using *Inveon Acquisition Workplace* software version 1.5 using an ordered-set expectation maximization three-dimensional maximum a posteriori algorithm with the following parameters: matrix, $256 \times 256 \times 159$; pixel size, $0.43 \times 0.43 \times 0.8 \text{ mm}^3$; and β value of 1.5 with uniform variance.

Statistical Analysis

Statistical analyses were performed using *SPSS* software version 16.0 (SPSS Inc, Chicago, IL) and *GraphPad Prism* version 4.00 for Windows. Differences in uptake of radiolabeled peptides were tested for significance using the nonparametric Mann-Whitney test. A p value below .05 was considered significant.

Results

IC_{50} Values

Table 2 shows the apparent IC_{50} values of the labeled peptides as determined in competitive binding assays. DOTA-sCCK8, labeled with ^{115}In or ^{69}Ga , displayed the highest affinity (0.77 and 0.83 nM, respectively), whereas the synthetic peptide sCCK8[$\text{Phe}^2(p\text{-CH}_2\text{SO}_3\text{H})$, $\text{Nle}^{3,6}$]

Table 2. IC₅₀ Values of ¹¹⁵In- and ⁶⁹Ga-Labeled DOTA Peptides (nM)

Peptide	¹¹⁵ In Labeled	⁶⁹ Ga Labeled
DOTA-sCCK8	0.77 ± 0.02	0.83 ± 0.08
DOTA-sCCK8[Phe ² (<i>p</i> -CH ₂ SO ₃ H), Nle ^{3,6}]	16.72 ± 0.08	16.54 ± 0.06
DOTA-MG0	7.26 ± 0.05	5.91 ± 0.06

IC₅₀ = 50% inhibitory concentration.

showed the lowest affinity (16.72 and 16.54 nM, respectively). No significant differences in IC₅₀ values were found between ⁶⁹Ga-labeled peptide and ¹¹⁵In-labeled peptides.

Biodistribution Studies of Radiolabeled DOTA Peptides

The in vivo characteristics of the radiolabeled peptides were studied in mice with A431-CCK2R and A431-mock tumors. The biodistribution of the DOTA peptides, labeled with ⁶⁸Ga and ¹¹¹In, for in vivo targeting of CCK2R-expressing tumors was determined at 1 hour (⁶⁸Ga and ¹¹¹In) and 4 hours (¹¹¹In) postinjection. The biodistribution profile for the ¹¹¹In-labeled peptides is summarized in Figure 1.

All three peptides showed specific targeting of the A431-CCK2R tumors. ¹¹¹In-DOTA-sCCK8 showed high uptake in the CCK2R-expressing tumor at 1 hour postinjection (9.01 ± 1.75% ID/g). Tumor uptake at 4 hours postinjection was lower (5.74 ± 0.76% ID/g, *p* = .01). Uptake of the synthetic peptide ¹¹¹In-DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] in the CCK2R-expressing tumor was significantly lower (4.95 ± 0.62% ID/g at 1 hour postinjection, *p* < .01) but showed a good tumor retention (5.44 ± 0.41% ID/g at 4 hours postinjection).

¹¹¹In-DOTA-MG0 showed a higher uptake in the tumor (12.5 ± 2.88% ID/g at 1 hour postinjection) than both sCCK8 peptides. For both sCCK8 peptides, specific uptake was also found in the pancreas and the stomach, presumably due to expression of the CCK1 receptor in these organs in rodents.

Kidney uptake of ¹¹¹In-DOTA-MG0 was high (70.3 ± 4.50% ID/g), whereas the sCCK8 peptides showed significantly lower uptake in the kidneys (sCCK8 1.46 ± 0.39% ID/g, sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] 1.20 ± 0.10% ID/g, both *p* < .001). For all peptides tested, blood levels and uptake in the lungs and peripheral soft tissues at 1 hour postinjection were negligible (< 1.0% ID/g).

In Figure 2, the biodistribution profile of the ⁶⁸Ga-labeled peptides is summarized.

Tumor uptake of ⁶⁸Ga-DOTA-MG0 (14.2 ± 4.06 %ID/g) was twofold higher than that of the ⁶⁸Ga-labeled sCCK8 peptides. Tumor uptake of ⁶⁸Ga-DOTA-sCCK8 was 7.48 ± 0.42% ID/g and that of ⁶⁸Ga-DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] was 6.35 ± 1.41% ID/g. Uptake of ⁶⁸Ga-DOTA-sCCK8 in the receptor-positive pancreas was higher than the tumor uptake. For ⁶⁸Ga-DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}], tumor uptake and pancreatic uptake were in the same range (6.35 ± 1.41% ID/g and 6.49 ± 0.33% ID/g, respectively).

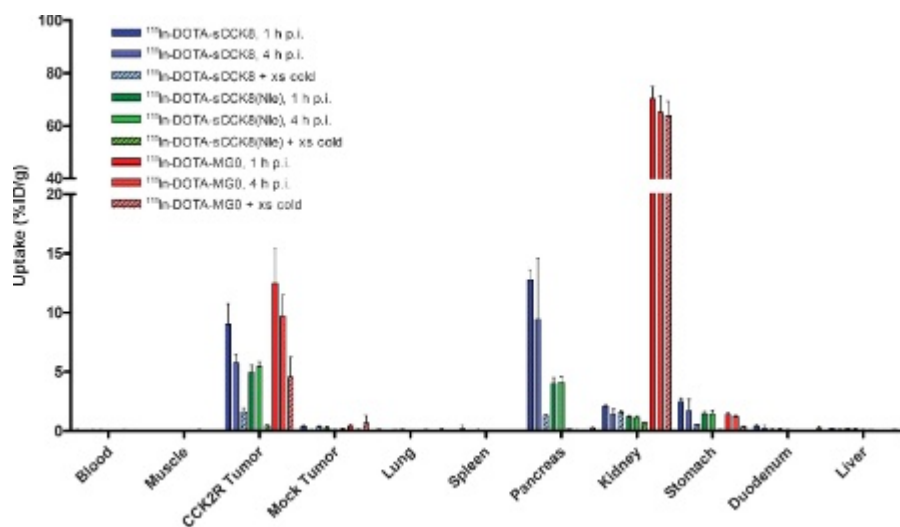


Figure 1. Biodistribution of ¹¹¹In-labeled peptides in BALB/c nude mice bearing CCK2R-expressing tumors. Values are expressed as a percentage of the injected dose per gram of tissue (*n* = 5 mice/group). Blocking was performed by coinjection of a 1,000-fold molar excess of unlabeled DOTA-sCCK8 (*n* = 3 mice/group). Mice were dissected 1 or 4 hours postinjection.

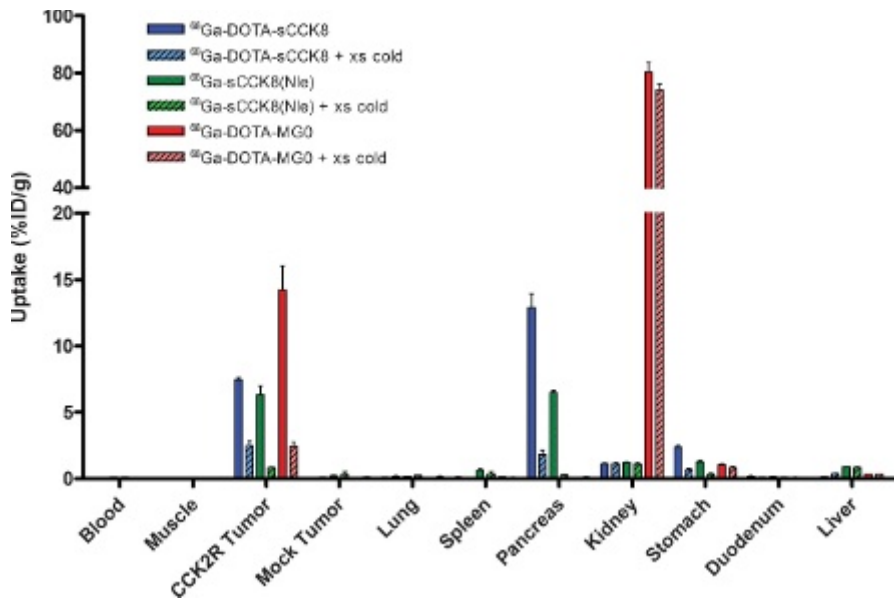


Figure 2. Biodistribution of ^{68}Ga -labeled peptides in BALB/c nude mice bearing CCK2R-expressing tumors. Values are expressed as a percentage of the injected dose per gram of tissue ($n = 5$ mice/group). Blocking was performed by coinjection of a 1,000-fold molar excess of unlabeled DOTA-sCCK8 ($n = 3$ mice/group). Mice were dissected 1 hour postinjection.

Kidney uptake of ^{68}Ga -DOTA-MG0 was $80.4 \pm 7.68\%$ ID/g, about 70-fold higher than the kidney uptake of the two sCCK8 peptides. Blood levels and uptake in normal tissues were low.

SPECT and PET

Subcutaneous A431-CCK2R tumors were clearly visualized on SPECT images 1 hour after injection of ^{111}In -labeled

DOTA peptides (Figure 3). For anatomic correlation, the SPECT images were fused with CT images. The A431-mock tumor did not show uptake with any of the peptides and thus was not visualized.

^{111}In -DOTA-sCCK8 showed some uptake in the abdomen, presumably due to accretion of the peptide in the pancreas. With ^{111}In -DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}], the stomach was also visualized. Tumor uptake of the synthetic peptide was somewhat lower than that of

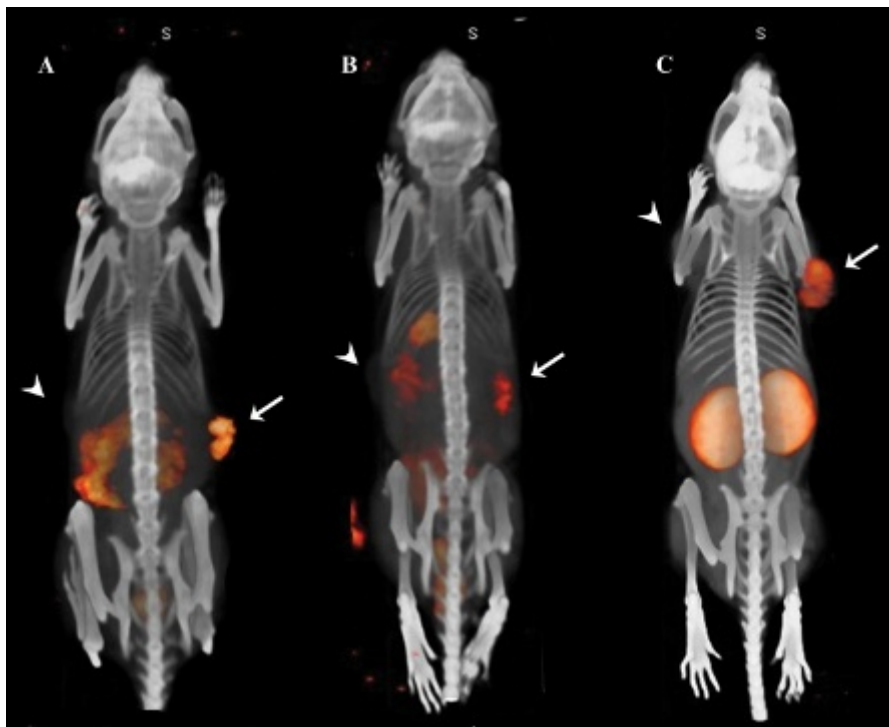


Figure 3. SPECT/CT images of mice with subcutaneous A431-CCK2R tumors 1 hour after injection of ^{111}In -labeled (A) DOTA-sCCK8, (B) DOTA-sCCK8 [Phe²(*p*-CH₂SO₃H), Nle^{3,6}], and (C) DOTA-MG0. Radiotracer uptake is clearly visible in A431-CCK2R tumors (arrow), whereas no uptake is observed in the mock-transfected A431 tumors (arrowhead). CCK2R to mock tumor ratios were 12.2, 7.9, and 27.5 for DOTA-sCCK8, DOTA-sCCK8 [Phe²(*p*-CH₂SO₃H), Nle^{3,6}], and DOTA-MG0, respectively.

the other two peptides. In line with the biodistribution results, pronounced uptake of ^{111}In -DOTA-MG0 was observed in the kidneys.

PET also allowed for clear visualization of the A431-CCK2R tumors 1 hour after injection of ^{68}Ga -labeled DOTA peptides (Figure 4). Besides tumor uptake, both sCCK8 and sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] showed intestinal uptake, which was most pronounced for sCCK8. Excretion of ^{68}Ga -DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] was observed in the bladder. As was seen in the biodistribution studies and on the PET images, ^{68}Ga -DOTA-MG0 showed high uptake in the kidneys.

Discussion

In earlier studies, it was found that various gastrin analogues (such as MG0) show good tumor targeting, accompanied by high kidney uptake.^{7,8} In contrast, sCCK8 peptides have shown low kidney uptake and better tumor to kidney ratios.^{5,9} In this study, we have characterized the *in vitro* and *in vivo* properties of CCKR binding peptides, MG0 and two sCCK8 peptides. DOTA-MG0 was used as a reference peptide because this peptide has been studied most extensively.^{2,3,7,10,11} To this end, the peptides were labeled with ^{111}In (gamma emitter) and ^{68}Ga (positron emitter) and evaluated for detection of CCK2R-positive tumors with SPECT and PET, respectively.

Competitive binding assays on A431-CCK2R cells revealed that all evaluated compounds have affinities for the CCK2R in the low nanomolar range. Labeled DOTA-sCCK8 had the highest affinity, whereas the synthetic peptide DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] showed a significantly lower affinity. However, labeled with ^{68}Ga , the tumor uptake of both compounds was similar. The biodistribution studies with the ^{111}In -labeled peptides showed that 4 hours postinjection, tumor uptake was maintained for DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}], whereas tumor uptake of the DOTA-sCCK8 and DOTA-MG0 decreased over time. This improved tumor retention of DOTA-sCCK8[Phe²(*p*-CH₂SO₃H), Nle^{3,6}] may be due to the enhanced resistance against oxidation of this peptide compared to DOTA-MG0 and DOTA-sCCK8.

PET is advantageous over conventional scintigraphy and SPECT because of its high sensitivity in combination with improved spatial resolution. However, SPECT tracers are less expensive than PET tracers because different gamma-emitting nuclides can be used, which are mostly longer-lived and more easy to obtain than PET tracers.

On the PET images in this study, tumors were visualized with higher contrast than on the SPECT images. Both SPECT and PET images showed uptake of radio-labeled sCCK8 peptides in the intestines, presumably due to the accretion of tracer in the pancreas.

Earlier research showed that ^{68}Ga -DOTA-MG0 is a suitable PET tracer for the detection of CCK2R-positive

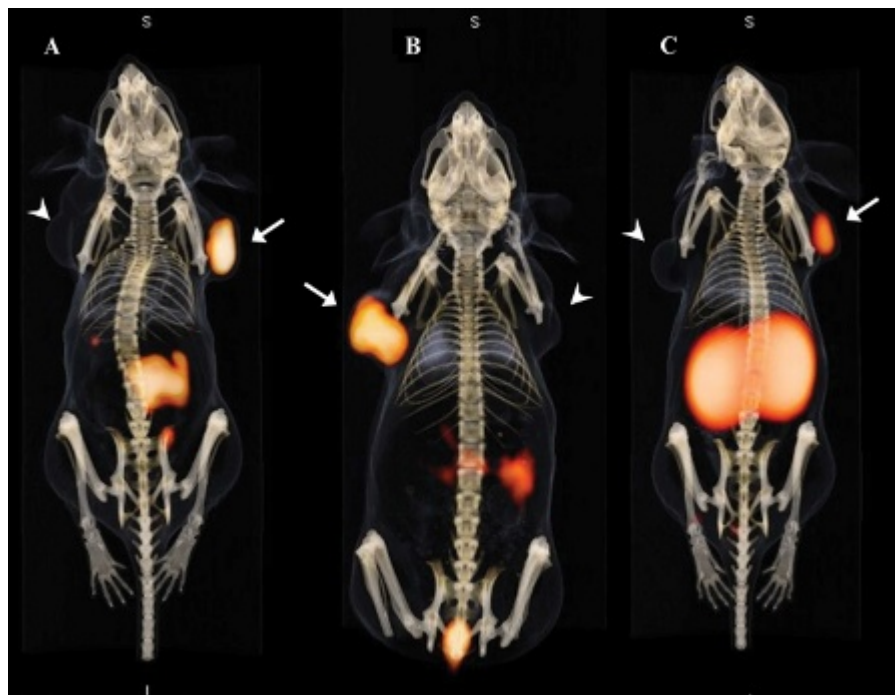


Figure 4. PET/CT images of mice with subcutaneous A431-CCK2R tumors 1 hour after injection of ^{68}Ga -labeled (A) DOTA-sCCK8, (B) DOTA-sCCK8 [Phe²(*p*-CH₂SO₃H), Nle^{3,6}], and (C) DOTA-MG0. Radiotracer uptake is clearly visible in A431-CCK2R tumors (arrow), whereas no uptake is observed in the mock-transfected A431 tumors (arrowhead). CCK2R to mock tumor ratios were 36.8, 5.4, and 15.0 for DOTA-sCCK8, DOTA-sCCK8 [Phe²(*p*-CH₂SO₃H), Nle^{3,6}], and DOTA-MG0, respectively.

tumors.¹⁰ However, the high kidney uptake might hamper the visualization of tumors in the vicinity of the kidneys. In this study, high kidney uptake is confirmed for DOTA-MG0 by PET, by SPECT, and in the biodistribution studies. This may be due to tubular reabsorption of the peptide via scavenger receptors.^{7,11} Béhé and colleagues showed that kidney uptake of radiolabeled DOTA-MG0 could be reduced by coadministration of polyglutamic acids.⁷ Recently, it was found that replacement of the penta-L-glutamate sequence by five D-glutamic acid or D-glutamine residues significantly lowered the kidney uptake, without affecting the tumor uptake.^{12,13} In addition, a peptide with only 3 N-terminal D-Gln also displayed a strongly reduced kidney retention, but its in vitro serum stability was less than that of the other analogues.¹³ The sCCK8 peptides also accumulated specifically in the pancreas. This specific uptake can be explained by the fact that in rodents, the pancreas abundantly expresses CCK1R and CCK2R, whereas in human pancreatic tissue, CCK2R is not expressed. Therefore, in a clinical setting, pancreatic uptake of radiolabeled sCCK8 analogues is not expected.^{5,9} Given that both sCCK8 peptides show low kidney uptake and good tumor retention, they might be promising tracers for imaging of CCK2R-positive tumors.

Conclusion

All three peptides showed CCK2R affinity in the low nanomolar range and good tumor uptake in CCK2R-positive tumors. Both ¹¹¹In-labeled and ⁶⁸Ga-labeled DOTA peptides showed high uptake in subcutaneous A431-CCK2R tumors. Subcutaneous tumors were clearly visualized with microPET after injection of ⁶⁸Ga-labeled DOTA peptides. In this study, DOTA-sCCK8 combines a high affinity with high tumor uptake and low kidney uptake. The synthetic peptide DOTA-sCCK8[Phe²(p-CH₂SO₃H), Nle^{3,6}] showed the best tumor retention, but the uptake is lower. Radiolabeled DOTA-MG0 showed the highest uptake in the tumor, but its high kidney uptake is disadvantageous. Our findings may serve as a stimulus to perform more research on the potential of sCCK8 analogues in visualizing CCK2R-positive tumors.

Acknowledgments

Bianca Lemmers and Kitty Lemmens are kindly acknowledged for their assistance with the animal experiments.

Financial disclosure of authors: This study was financially supported by Dutch Cancer Society grant KUN 2006-3575. Dr. Luigi Aloj (Istituto Nazionale Tumori, Fondazione G. Pascale, Naples, Italy) is gratefully acknowledged for providing the transfected A431 cells.

Financial disclosure of reviewers: None reported.

References

1. Reubi JC, Schaer JC, Waser B. Cholecystokinin(CCK)-A and CCK-B/gastrin receptors in human tumors. *Cancer Res* 1997;57:1377–86.
2. Behr TM, Jenner N, Béhé MP, et al. Radiolabeled peptides for targeting cholecystokinin-B/gastrin receptor-expressing tumors. *J Nucl Med* 1999;40:1029–44.
3. Mather SJ, McKenzie AJ, Sosabowski JK, et al. Selection of radiolabeled gastrin analogs for peptide receptor-targeted radionuclide therapy. *J Nucl Med* 2007;48:615–22, doi:10.2967/jnumed.106.037085.
4. Breeman WAP, Fröberg AC, de Blois E, et al. Optimised labeling, preclinical and initial aspects of CCK-2 receptor-targeting with 3 radiolabeled peptides. *Nucl Med Biol* 2008;35:839–49, doi:10.1016/j.nucmedbio.2008.09.006.
5. Roosenburg S, Laverman P, Joosten L, et al. Synthesis and evaluation of ¹¹¹In-labeled sCCK8 analogues for targeting CCK2-receptor positive tumors. *Bioconjug Chem* 2010;21:663–70, doi:10.1021/bc900465y.
6. Aloj L, Caraco C, Panico M, et al. In vitro and in vivo evaluation of In-111-DTPAGlu-G-CCK8 for cholecystokinin-B receptor imaging. *J Nucl Med* 2004;45:485–94.
7. Béhé MP, Kluge G, Becker W, et al. Use of polyglutamic acids to reduce uptake of radiometal-labeled minigastrin in the kidneys. *J Nucl Med* 2005;46:1012–5.
8. Nock BA, Maina T, Béhé MP, et al. CCK-2/gastrin receptor-targeted tumor imaging with ^{99m}Tc-labeled minigastrin analogs. *J Nucl Med* 2005;46:1727–36.
9. Laverman P, Béhé MP, Oyen WJG, et al. Two technetium-99m-labeled cholecystokinin-8 (CCK8) peptides for scintigraphic imaging of CCK receptors. *Bioconjug Chem* 2004;15:561–8, doi:10.1021/bc034208w.
10. Brom M, Joosten L, Laverman P, et al. Preclinical evaluation of ⁶⁸Ga-DOTA-minigastrin for the detection of cholecystokinin-2/gastrin receptor-positive tumors. *Mol Imaging* 2011;10:144–52.
11. Vegt E, van Eerd JE, Eek A, et al. Reducing renal uptake of radiolabeled peptides using albumin fragments. *J Nucl Med* 2008;49:1506–11, doi:10.2967/jnumed.108.053249.
12. Laverman P, Joosten L, Eek A, et al. Comparative biodistribution of twelve ¹¹¹In-labelled gastrin/CCK2 receptor-targeting peptides. *Eur J Nucl Med Mol Imaging* 2011;38:1410–6, doi:10.1007/s00259-011-1806-0.
13. Kolenc-Peittl PK, Mansi R, Tamma M, et al. Highly improved metabolic stability and pharmacokinetics of indium-111-DOTA-gastrin conjugates for targeting of the gastrin receptor. *J Med Chem* 2011;54:2602–9, doi:10.1021/jm101279a.

Copyright of Molecular Imaging is the property of Decker Publishing and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.