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PET of Tumors Expressing Gastrin-Releasing Peptide Receptor with an ^{18}F-Labeled Bombesin Analog

Ingrid Dijkgraaf[1], Gerben M. Franssen[1], William J. McBride[2], Christopher A. D'Souza[2], Peter Laverman[1], Charles J. Smith[3,4], David M. Goldenberg[2,5], Wim J.G. Oyen[1], and Otto C. Boerman[1]

[1]Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [2]Immunomedics, Inc., Morris Plains, New Jersey; [3]University of Missouri, School of Medicine, Columbia, Missouri; [4]Harry S. Truman Memorial Veterans' Hospital, Columbia, Missouri; and [5]Garden State Cancer Center, Center for Molecular Medicine and Immunology, Morris Plains, New Jersey

The gastrin-releasing peptide receptor (GRPR) is overexpressed in human prostate cancer. Bombesin (BBN) is a neurotransmitter of 14 amino acids and binds with selectivity and with high affinity to GRPRs. We have synthesized a NOTA-conjugated bombesin derivative, NOTA-8-Aoc-BBN(7-14)NH_2, to label this analog with ^{18}F using the new Al^{18}F method. In this study, the GRPR-targeting potential of ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2 was studied using ^{68}Ga-NOTA-8-Aoc-BBN(7-14)NH_2 as a reference. **Methods:** The NOTA-conjugated bombesin analog was synthesized and radiolabeled with ^{68}Ga or ^{18}F. For ^{18}F labeling, we used our new 1-pot, 1-step method. The labeled product was purified by reversed-phase high-performance liquid chromatography. The log P values of the radiotracers were determined. The tumor-targeting characteristics of the compounds were assessed in mice with subcutaneously growing PC-3 xenografts. GRPR-binding specificity was studied by coinjection of an excess of unlabeled NOTA-8-Aoc-BBN(7-14)NH_2. Small-animal PET/CT images were acquired. **Results:** NOTA-8-Aoc-BBN(7-14)NH_2 could be efficiently labeled with ^{18}F or with ^{68}Ga. NOTA-8-Aoc-BBN(7-14)NH_2 was labeled with ^{18}F in a single step, with 50%–90% yield. Radiolabeling, including purification, was performed in 45 min and resulted in a specific activity of greater than 10 GBq/μmol. The log P values of ^{18}F- and ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 were -1.47 ± 0.05 and -1.98 ± 0.03, respectively. In mice, both radiolabeled compounds cleared rapidly from the blood (<0.07 percentage injected dose per gram at 1 h after injection), mainly via the kidneys. At 1 h after injection, the uptake of ^{18}F- and ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 in the PC-3 tumors was 2.15 ± 0.55 and 1.24 ± 0.26 percentage injected dose per gram, respectively. GRPR-binding specificity was demonstrated by reduced tumor uptake of radiolabeled NOTA-8-Aoc-BBN(7-14)NH_2 after coinjection of a 100-fold excess of unlabeled NOTA-8-Aoc-BBN(7-14)NH_2 peptide. The accumulation of ^{18}F-NOTA-8-Aoc-BBN(7-14)NH_2 in the subcutaneous PC-3 tumors could be visualized via small-animal PET. **Conclusion:** NOTA-8-Aoc-BBN(7-14)NH_2 could be labeled rapidly and efficiently with ^{18}F using a 1-pot, 1-step method. Radiolabeled NOTA-8-Aoc-BBN(7-14)NH_2 specifically accumulated in the GRPR-expressing PC-3 tumors and should be evaluated clinically.

Key Words: GRPR; bombesin; PET; radiofluorination; aluminum chloride

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Prostate cancer is the third-leading cause of cancer-related deaths and the most frequently diagnosed cancer among men in the Western world (*1*). There is a great need for an imaging technique that provides adequate staging of patients with this disease. A promising approach to cancer diagnosis is the use of radiolabeled receptor-binding peptides. It has been shown that prostate tumors overexpress the gastrin-releasing peptide receptor (GRPR) (*2–6*). GRPRs are expressed in primary prostate cancer, invasive prostate carcinoma, and prostatic intraepithelial neoplasms at high density, whereas normal prostate tissue and benign prostate hyperplasia (BPH) are predominantly GRPR-negative (2). These findings suggest that the GRPR represents an ideal molecular target for radiolabeled GRPR ligands for diagnosis and staging of prostate cancer.

Bombesin (BBN) is the 14-amino acid amphibian peptide analog of the 27-amino acid mammalian gastrin-releasing peptide (GRP). BBN and GRP share a homologous 7–amino acid amidated C-terminus, Trp-Ala-Val-Gly-His-Leu-Met-NH_2, which is necessary for binding to the GRPR. Because GRP suffers from poor in vivo stability, developments have focused on BBN analogs. Synthetic BBNs are truncated versions of BBNs containing the sequence or a full-length 14–amino acid sequence in which one or more amino acids have been replaced. The C-terminus of BBN is required for high-affinity binding and biologic potency (*7*). Therefore, the N-terminus of BBN is usually modified for labeling the analogs with radioisotopes. A variety of BBN analogs has been developed and labeled with various radionuclides, such as ^{99m}Tc and ^{111}In, for SPECT (*8*) and ^{64}Cu, ^{68}Ga, or ^{86}Y for PET (*9,10*). ^{18}F is the most widely used radionuclide in PET. ^{18}F has excellent

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For correspondence or reprints contact: Otto C. Boerman, Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.
E-mail: O.Boerman@nucmed.umcn.nl
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characteristics for peptide-based receptor imaging studies: the half-life (109.7 min) matches the pharmacokinetics of most peptides, and the low positron energy of 635 keV results in a short penetration range in tissue and excellent imaging resolution (*11*). Several methods have been developed to label peptides with ^{18}F. However, in general these methods are laborious and require a multistep synthesis. Recently, McBride et al. reported a facile method wherein ^{18}F is first attached to aluminum as Al^{18}F, which is then complexed in a chelating agent attached to the peptide, forming a stable Al^{18}F–chelate peptide complex in an efficient 1-pot process (*12*).

Several studies have indicated that the DOTA-conjugated BBN analog, DOTA-8-Aoc-BBN(7-14)NH_2, satisfies inherent in vitro and in vivo requirements for radiopharmaceutical development (*13–15*). However, the DOTA chelator in the studies of Rogers et al. (*13*) showed demetallation of ^{64}Cu. Therefore, Prasanphanich et al. conjugated the NOTA chelator to 8-Aoc-BBN(7-14)NH_2 to produce a kinetically inert BBN conjugate (*16*).

As shown previously, NOTA-conjugated peptides can be labeled with ^{18}F using the Al^{18}F method (*17–19*). Here, we describe the ^{18}F labeling of NOTA-8-Aoc-BBN(7-14)NH_2, using the new 1-pot, 1-step, labeling method. The in vitro affinity and the in vivo tumor targeting characteristics of ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2 were determined and compared with those of the ^{68}Ga-labeled counterpart.

MATERIALS AND METHODS

Synthesis of NOTA-Conjugated BBN Derivative 8-Aoc-BBN(7-14)NH_2

The BBN derivative was synthesized using Fmoc-based solid-phase peptide synthesis as described previously (*16*). The structural formula of NOTA-8-Aoc-BBN(7-14)NH_2 is shown in Figure 1.

Radiolabeling

Labeling with ^{18}F. NOTA-8-Aoc-BBN(7-14)NH_2 was radiolabeled with ^{18}F as described by Laverman et al. (*17*). Briefly, a Chromafix cartridge with 2–6 GBq of ^{18}F (BV Cyclotron VU) was washed with 3 mL of metal-free water. ^{18}F was eluted from the cartridge with 100 μL of 0.4 M $KHCO_3$. The pH of the eluate was adjusted to 4.1 with 5 μL of metal-free glacial acetic acid, and 2–40 μL of 2 mM $AlCl_3$ in 0.1 M sodium acetate buffer (pH 4.1) were added. Finally, 80 μL of this Al^{18}F solution was added to 400 μL of MeCN and 20 μL of NOTA-8-Aoc-BBN(7-14)NH_2 (5 μg/μL) in 0.5 M sodium acetate, pH 4.1. The reaction mixture was heated at 100°C for 15 min. The radiolabeled peptide was purified by reversed-phase high-performance liquid chromatography (RP-HPLC) using the gradient as in the "Quality Control" section. Fractions containing ^{18}F-NOTA-8-Aoc-BBN(7-14)NH_2 were collected and diluted 10-fold with H_2O and purified on an Oasis HLB 1-cm^3 (10-mg) cartridge (Waters) to remove acetonitrile and trifluoroacetic acid. In brief, the fraction was applied on the cartridge, and the cartridge was washed with 3 × 1 mL of H_2O. The radiolabeled peptide was eluted with 200 μL of EtOH/H_2O (1:1, v/v). For injection into mice, the peptide was diluted with 0.5% bovine serum albumin in 0.9% NaCl.

Labeling with ^{68}Ga. ^{68}Ga was obtained from a 1,850-MBq ^{68}Ge/^{68}Ga generator (IGG-100, Eckert & Ziegler), where ^{68}Ge was attached to a TiO_2-based column. The ^{68}Ga was eluted with 0.1 M HCl (Ultrapure; J.T. Baker) using an Econo Pump (Bio-Rad) at 1 mL/min. Five 1-mL fractions were collected, and an aliquot of the second fraction was used for labeling the peptide.

^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 was prepared by adding 20 μL of 2.5 M HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) solution to 5 μL of the peptide dissolved in 2.5 M HEPES (2 μg/μL). Then, the second fraction from the ^{68}Ge/^{68}Ga generator (315–365 MBq) was added. After 10 min at 95°C, the ^{68}Ga-labeled peptide was purified on an Oasis HLB (30-mg) cartridge (Waters). After the sample was applied to the cartridge, the cartridge was washed with 3 × 1 mL of H_2O and eluted with 200 μL of EtOH/H_2O (1:1, v/v).

Quality Control. The radiolabeled preparations were analyzed by RP-HPLC on an Agilent 1200 system (Agilent Technologies). A C18 column (Onyx monolithic, 4.6 mm × 100 mm; Phenomenex) was used at a flow rate of 3 mL/min with the following buffer system: buffer A, 0.1% v/v trifluoroacetic acid in H_2O; buffer B, 0.1% v/v trifluoroacetic acid in acetonitrile; and a gradient of 80% buffer A at 0–5 min and 80% buffer A to 76% buffer A at 5–20 min. The radioactivity of the eluate was monitored using an in-line NaI radiodetector (Raytest GmbH). Elution profiles were analyzed using Gina-star software (version 2.18; Raytest GmbH). For injection into mice, the peptide was diluted to less than 10% (v/v) ethanol with 0.5% (w/v) bovine serum albumin in 0.9% NaCl.

Octanol/Water Partition Coefficient

To an Eppendorf tube filled with 0.5 mL of the radiolabeled peptide in phosphate-buffered saline (pH 7.4), 0.5 mL of octanol was added. After the tube was vigorously stirred by a vortex mixer for 2 min at room temperature, the 2 layers were separated by centrifugation (100*g*,

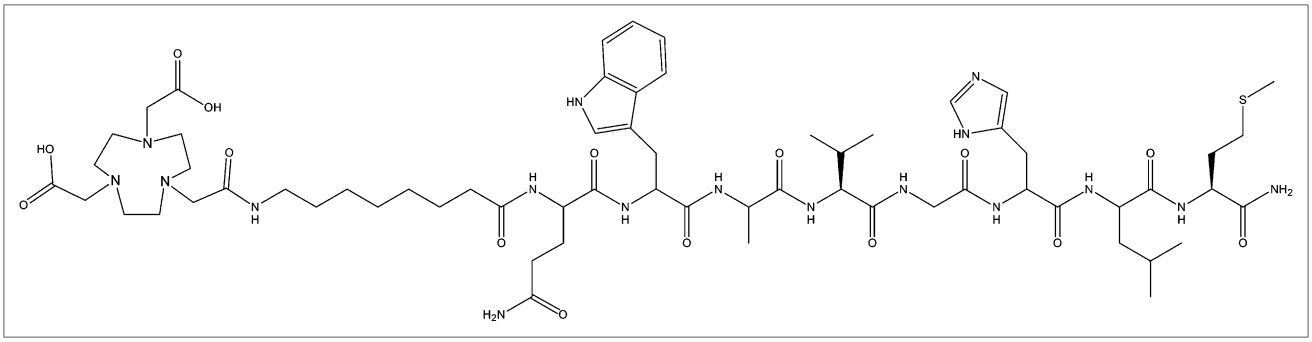


FIGURE 1. Structural formula of NOTA-conjugated BBN analog NOTA-8-Aoc-BBN(7-14)NH_2.

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5 min). Samples of 100 μL were taken from each layer, radioactivity was measured in a well-type γ-counter (Wallac Wizard 3″; Perkin-Elmer), and log P values were calculated ($n = 3$).

Competitive Cell-Binding Assay

The PC-3 human prostate cancer cell line was cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum. Cells were grown in tissue culture flasks at 37°C in a humidified atmosphere containing 5% CO_2 and routinely passed using 0.25% trypsin/ethylenediaminetetraacetic acid.

Binding affinities toward the GRPR for ^{nat}Ga-, Al^{nat}F-, and nonlabeled NOTA-8-Aoc-BBN(7-14)NH_2 were determined in a competitive binding assay on PC-3 tumor cells, using ^{111}In-NOTA-8-Aoc-BBN(7-14)NH_2 as a tracer. PC-3 cells were seeded into 6-well plates at 3×10^5 cells per well and cultured until confluency. On the day of the assay, cells were washed with binding buffer (RPMI, 0.5% bovine serum albumin). Subsequently, binding buffer (1.5 mL), ^{nat}Ga-, Al^{nat}F-, or nonlabeled NOTA-8-Aoc-BBN(7-14)NH_2 in a range of 0.1 to 300 nM and 200 Bq of ^{111}In-NOTA-8-Aoc-BBN(7-14)NH_2 were added to each well. After incubation for 3 h, medium was removed. Then, cells were washed with binding buffer and extracted from the wells, and cell-associated radioactivity was determined in a γ-counter. For the complexation of NOTA-8-Aoc-BBN(7-14)NH_2 with $^{nat}Ga^{3+}$, the peptide was dissolved in an aqueous solution (2.5 M HEPES; 1 μg/μL) and a 3-fold molar excess of $^{nat}Ga(NO_3)_3$ (Sigma-Aldrich Chemie) in 0.1N HCl solution was added. Metal complexation was performed overnight at room temperature. Al^{nat}F complexation of NOTA-8-Aoc-BBN(7-14)NH_2 was performed by mixing an AlF solution (0.02 M $AlCl_3$ in 0.5 M NaAc, pH 4, with 0.1 M NaF in 0.5 M NaAc, pH 4.1) with the NOTA-conjugated peptide. This reaction mixture was heated for 15 min at 100°C and then purified by RP-HPLC. GraphPad Prism software (version 5.03 for Windows [Microsoft]; GraphPad Software) was used to calculate inhibitory concentration of 50% (IC_{50}) values.

Biodistribution Studies

Male nude BALB/c mice (6–8 wk old) were injected subcutaneously in the right flank with 0.2 mL of a PC-3 cell suspension of 1.5×10^7 cells/mL (66% RPMI, 33% Matrigel [BD Biosciences]). Two weeks after inoculation of the tumor cells, mice were injected intravenously with the ^{18}F- or ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 (<1 μg). Mice were killed by CO_2 asphyxiation 1 h after injection (5–6 mice per group). Blood, tumor, and the major organs and tissues were collected, weighed, and counted in a γ-counter. The percentage injected dose per gram (%ID/g) was determined for each sample.

The receptor-mediated localization of the radiolabeled peptide was investigated by determining the biodistribution of the ^{18}F- or ^{68}Ga-labeled peptide after coinjection of an excess of unlabeled peptide ($n = 3$). All animal experiments were approved by the local Animal Welfare Committee in accordance with Dutch legislation and performed in accordance with their guidelines.

Small-Animal PET/CT

Mice with subcutaneous PC-3 tumors were injected intravenously with 2 MBq of ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2. One hour after the injection of the radiolabeled peptide, mice were scanned on an animal PET/CT scanner (Inveon; Siemens Preclinical Solutions) with an intrinsic spatial resolution of 1.5 mm (*11*). The animals were placed supine in the scanner. PET emission scans were acquired over 20–25 min, followed by a CT scan for anatomic reference (spatial resolution, 113 μm; 80 kV; and 500 μA). Scans were reconstructed using Inveon Acquisition Workplace software (version 1.5; Siemens Preclinical Solutions), using a 3-dimensional ordered-subset expectation maximization/maximum a posteriori algorithm with the following parameters: matrix, $256 \times 256 \times 159$; pixel size, $0.43 \times 0.43 \times 0.8$ mm; and β-value, 1.5, with uniform variance

Statistical Analysis

All mean values are given with SD. Statistical analysis was performed using a Welch corrected unpaired Student *t* test or 1-way ANOVA using GraphPad InStat software (version 3.06; GraphPad Software). The level of significance was set at *P* less than 0.05.

RESULTS

Radiolabeling

RP-HPLC analysis indicated that the radiochemical purity of the ^{18}F- or ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 preparations used in these experiments always exceeded 95%. Radio-HPLC chromatograms of ^{18}F- and ^{68}Ga-labeled NOTA-Aoc-BBN(7-14)NH_2 are shown in Figure 2. Al^{18}F-NOTA-Aoc-BBN(7-14)NH_2 had a retention time of 16.3 min and eluted at 23% of buffer B. ^{68}Ga-NOTA-Aoc-BBN(7-14)NH_2 had a 12.7-min retention time and eluted at 22% of buffer B.

Octanol/Water Partition Coefficient

To establish lipophilicity of the ^{18}F- and ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2, the octanol/water partition coefficients were determined. The log $P_{octanol/water}$ value for the ^{18}F-labeled BBN analog was -1.47 ± 0.05. The ^{68}Ga-labeled analog was more hydrophilic, with a log $P_{octanol/water}$ value of -1.98 ± 0.03.

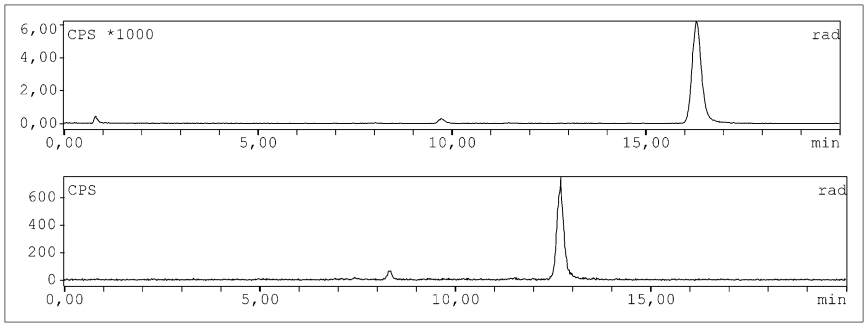


FIGURE 2. Radio-HPLC chromatograms of ^{18}F- (top) and ^{68}Ga-labeled (bottom) NOTA-8-Aoc-BBN(7-14)NH_2.

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Competitive Binding Assay

The affinity of Al^{nat}F-, ^{nat}Ga-, and nonlabeled NOTA-8-Aoc-BBN(7-14)NH_2 for GRPR was determined in a competitive binding assay. The results of these assays are summarized in Figure 3. Binding of ^{111}In-labeled BBN peptide to GRPR was displaced by ^{nat}Ga-, Al^{nat}F-, and nonlabeled NOTA-8-Aoc-BBN(7-14)NH_2 in a concentration-dependent manner. The IC_{50} values of the Al^{nat}F-, ^{nat}Ga-, and nonlabeled compounds were not significantly different and in the subnanomolar range (0.28 ± 0.15, 0.41 ± 0.15, and 0.37 ± 0.15 nM, respectively), indicating high binding affinity for the GRPR.

Biodistribution Studies

The results of the biodistribution studies of both ^{18}F- and ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 are summarized in Figure 4. NOTA-8-Aoc-BBN(7-14)NH_2 radiolabeled with either ^{18}F or ^{68}Ga cleared rapidly from the blood, in which levels of both tracers were below 0.07 %ID/g at 1 h after injection. Tumor uptake of ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2 was significantly higher than that of ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 (2.15 ± 0.55 vs. 1.24 ± 0.26 %ID/g, respectively; $P < 0.05$). In addition to tumor uptake, ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2 had significantly higher uptake in the pancreas than did the ^{68}Ga-labeled analog (27.09 ± 12.77 %ID/g vs. 5.93 ± 2.10 %ID/g; $P < 0.01$).

The coinjection of an excess of unlabeled NOTA-8-Aoc-BBN(7-14)NH_2 (50 μg) along with ^{18}F- or ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 resulted in a significantly reduced radioactivity concentration in the tumor, indicating that the major fraction of the uptake of radiolabeled NOTA-8-Aoc-BBN(7-14)NH_2 in the tumor was GRPR-mediated. Also, GRPR-specific uptake in the pancreas for ^{18}F- and ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2, in the presence of an excess of nonradiolabeled peptide showed significant reduction in accumulation of the tracer (0.43 ± 0.08 and 0.21 ± 0.09 %ID/g).

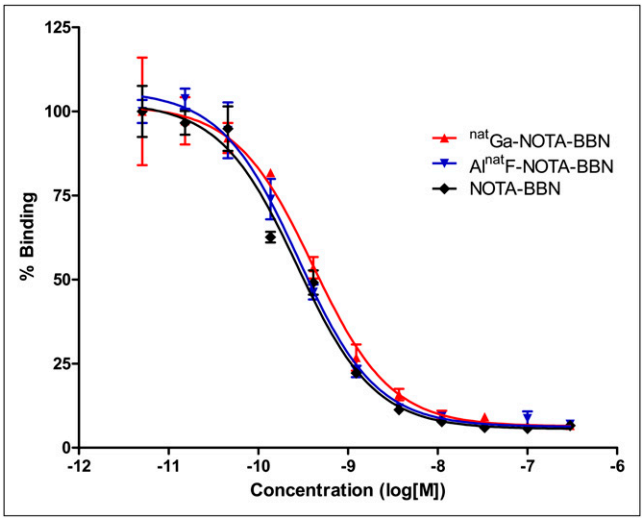


FIGURE 3. Competition of specific binding of ^{111}In-NOTA-8-Aoc-BBN(7-14)NH_2 with various NOTA-8-Aoc-BBN(7-14)NH_2 analogs.

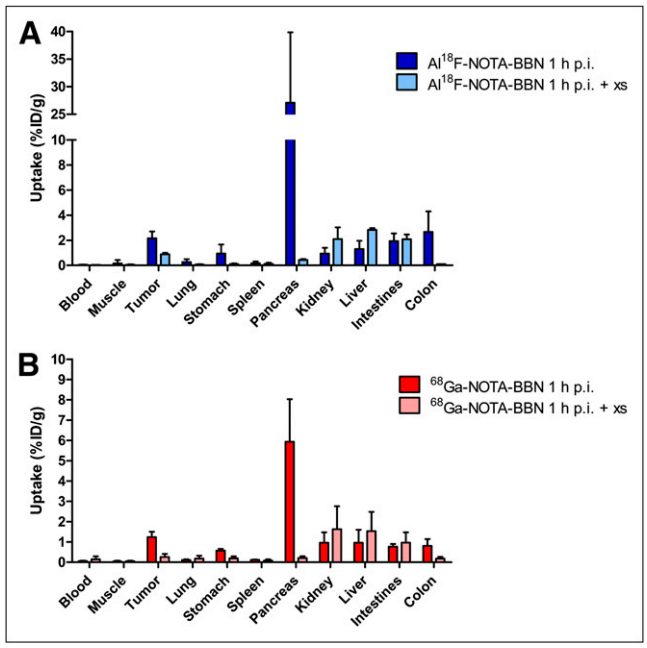


FIGURE 4. (A) Biodistribution of ^{18}F-NOTA-8-Aoc-BBN(7-14)NH_2 at 1 h after injection in athymic mice with subcutaneous PC-3 tumors in absence (5 mice per group) or presence (2 mice per group) of excess of nonradiolabeled NOTA-8-Aoc-BBN(7-14)NH_2. (B) Biodistribution of ^{68}Ga-NOTA-8-Aoc-BBN(7-14)NH_2 at 1 h after injection in athymic mice with subcutaneous PC-3 tumors in absence (6 mice per group) or presence (3 mice per group) of excess of nonradiolabeled NOTA-8-Aoc-BBN(7-14)NH_2. p.i. = after injection; xs = excess.

Small-Animal PET/CT

Fused PET and CT images are shown in Figure 5. PET scans were in line with the biodistribution data. PC-3 tumors could be clearly visualized. However, there was considerable accumulation and retention in other organs such as the kidneys, liver, and intestines. In addition, uptake in the pancreas was relatively high and appeared to be specific. As a whole, uptake in the abdominal cavity was relatively high and is not entirely unexpected.

DISCUSSION

The diagnostic accuracy of metabolic PET tracers, such as ^{18}F-FDG, ^{18}F- or ^{11}C-choline, and ^{11}C-acetate, for imaging prostate cancer is limited, because most prostate tumor lesions are characterized by a low metabolic activity (*20*). The discovery of GRPR overexpression in prostate cancer disease has led to the development and application of radiolabeled BBN derivatives for imaging and staging of prostate cancer using SPECT or PET. Here, the feasibility of using an ^{18}F-labeled BBN analog for radionuclide imaging of GRPR expression with PET was investigated. The NOTA-conjugated BBN analog was radiolabeled with ^{18}F, using the new chelator-based method developed by McBride et al. (*12*). The in vitro and in vivo characteristics of this ^{18}F-labeled peptide were directly compared with those of the ^{68}Ga-labeled analog.

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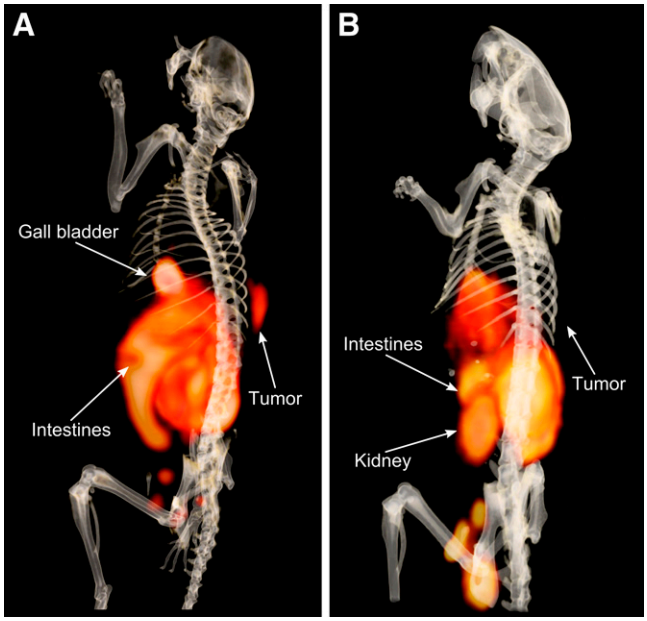


FIGURE 5. 3-dimensional volume–rendering projections of fused PET and CT scans of mice with subcutaneously growing PC-3 tumor after intravenous injection of ^{18}F-NOTA-8-Aoc-BBN(7-14)NH_2 in absence (A) or presence (B) of excess of nonradiolabeled NOTA-8-Aoc-BBN(7-14)NH_2. Scans were recorded at 1 h after injection.

To radiolabel NOTA-conjugated BBN peptide with ^{18}F, we used our optimized 1-pot method for labeling peptides with ^{18}F as described recently (*17*). First, $^{18}F^-$ was complexed with Al^{3+}, and subsequently the $Al^{18}F$ complex was chelated by NOTA. It is still unclear which aluminum–fluoride complex is formed, but because the formation of $Al^{18}F$ is performed at pH 4.1, we believe that the form in sodium acetate buffer is probably AlF^{2+}. In addition, the molar ratio of aluminum to ^{18}F is such that it is unlikely that any AlF_2^+ or AlF_3 is formed (*21*).

The ^{18}F-labeled peptide was slightly more lipophilic than the ^{68}Ga-labeled BBN analog, as indicated by their log P values (-1.98 ± 0.03 and -1.47 ± 0.05, respectively). This difference could be due to the dianionic NOTA chelator, which stabilizes the +2 charge of the $Al^{18}F$-complex but not the +3 charge of the ^{68}Ga atom, resulting in an overall +1 charge for the ^{68}Ga-NOTA complex. However, the presence of counter ions could play a role as well.

In a competitive binding assay using GRPR-expressing PC-3 cells, the IC_{50} values of the nonlabeled and cold labeled analogs were not significantly different and were in the subnanomolar range. Prasanphanich et al. also determined the IC_{50} value of NOTA-8-Aoc-BBN(7-14)NH_2, and in this assay the binding affinity of their compound was somewhat lower than the binding affinity as determined in our assay (3.1 ± 0.5 vs. 0.37 ± 0.2 nM) (*16*). Although they also used PC-3 cells in their assay, their tracer was ^{125}I-Tyr^4-BBN, whereas in our assay the ^{111}In-NOTA-8-Aoc-BBN(7-14)NH_2 ligand was used as the displacement radioligand.

In the subcutaneous PC-3 xenograft model, the tumor uptake of ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2 was significantly higher than that of ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 at 1 h after injection (2.15 ± 0.55 vs. 1.24 ± 0.26 %ID/g, respectively; $P < 0.05$). Both tracers showed specific tumor uptake in PC-3 tumors: the coinjection of an excess of unlabeled NOTA-8-Aoc-BBN(7-14)NH_2 resulted in a significantly lower tumor uptake of ^{68}Ga-NOTA-8-Aoc-BBN(7-14)NH_2 and ^{18}F-NOTA-8-Aoc-BBN(7-14)NH_2 (0.26 ± 0.15 and 0.88 ± 0.12 %ID/g, respectively). Several tissues in mice express the GRPR, including the small intestine, large intestine, and pancreas (*22*). In our study, specific uptake of ^{68}Ga-NOTA-8-Aoc-BBN(7-14)NH_2 and ^{18}F-NOTA-8-Aoc-BBN(7-14)NH_2 was found in the colon and pancreas, indicating that the accumulation of these tracers in these tissues is at least partly GRPR-mediated. ^{18}F- and ^{68}Ga-labeled NOTA-8-Aoc-BBN(7-14)NH_2 cleared rapidly from the blood, resulting in high tumor-to-blood ratios at 1 h after injection (49.51 ± 22.94 and 25.06 ± 10.55, respectively).

The uptake of the ^{64}Cu-labeled NOTA-8-Aoc-BBN (7-14) conjugate in PC-3–bearing mice (3.59 ± 0.70 % ID/g, 1 h after injection) was similar to the tumor uptake for our radiolabeled compounds (2.15–1.24.%ID/g, 1 h after injection) (*16*). However, Lears et al. recently reported in this tumor model a tumor uptake as high as 13.0 %ID/g at 1 h after injection using ^{64}Cu-labeled SarAr-SA-Aoc-BBN(7-14) (*23*). This compound has an additional linker and is labeled with another radionuclide via a different chelator, and each of these parameters could have caused the higher uptake of this tracer in the tumor. However, in our experiments we observed that the tumor uptake can vary largely from one experiment to the other. Even within the same experiment, the uptake of the radiolabeled BBN analog in the PC-3 tumor varied considerably.

To select the best ligand for clinical evaluation, Schroeder et al. performed a comparative study using 5 radiolabeled BBN analogs under identical experimental conditions (*24*). In previous studies in PC-3–bearing mice, ^{67}Ga-PESIN, ^{177}Lu-AMBA, and ^{99m}Tc-demobesin-1 showed tumor uptake of 14.8, 6.4, and 16.2 %ID/g, respectively (*9,25–28*). Variation in amounts of peptides, mouse strain (species, sex, and weight), tumor cells (passage number, culture conditions) used, tumor size, and vascularization of the tumor may all be factors that affect tumor uptake and tumor–to–non-tumor ratios. On the basis of these data, the authors selected ^{99m}Tc-demobesin-1 as the bombesin analog with optimal characteristics for prostate cancer imaging. This study of the group in Rotterdam demonstrated that the characteristics of radiolabeled BBN analogs can be appreciated only in a direct comparative study.

Overall, the biodistribution of ^{18}F-NOTA-8-Aoc-BBN (7-14)NH_2 in mice with subcutaneous PC-3 xenografts was similar to that of ^{68}Ga-NOTA-8-Aoc-BBN(7-14)NH_2, indicating that labeling the analog with $Al^{18}F$ did not affect the in vivo characteristics of the bombesin derivative.

The $Al^{18}F$ method is a fast (45 min) radiofluorination strategy and does not affect the pharmacokinetics of the bombesin peptide. The commonly used methodology for efficient ^{18}F-

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labeling of peptides consists of 2 steps: first the preparation of an ^{18}F-labeled synthon (prosthetic groups), which is subsequently conjugated to the peptide or the protein. In general, this fluorination is based on a nucleophilic substitution that requires laborious azeotropic drying of the ^{18}F-fluoride–kryptofix complex. The total synthesis and formulation time for these methods ranges between 1 and 3 h, with most of the time dedicated to the HPLC purification of the labeled peptides to obtain the carrier-free tracer required for in vitro and in vivo experiments. We also had to purify our ^{18}F-labeled peptide with HPLC to obtain a high-specific-activity preparation. Recently, McBride et al. have shown that the AlF method can be further optimized using other chelators (*19,29*). They developed a 1-pot method that provides a high labeling efficiency and specific activity with no more than only solid-phase extraction purification needed before formulation for injection. Because the Al^{18}F-method is based on a chelator-derivatized peptide, this labeling method is easy and versatile.

CONCLUSION

The Al^{18}F method combines the ease of chelator-based radiolabeling methods with the advantages of ^{18}F, such as half-life, availability, and positron energy. The ^{18}F-labeled NOTA-8-Aoc-BBN(7-14)NH_2 could be synthesized in less than 45 min without the need to synthesize a synthon. The results of the biodistribution study of the ^{18}F- and ^{68}Ga-labeled BBN peptide show that the AlF method does not affect the in vivo behavior of the analog. The ^{18}F-labeled BBN peptide is a suitable ligand for the noninvasive visualization of GRPR expression in vivo.

DISCLOSURE STATEMENT

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Ingrid Dijkgraaf, Gerben M. Franssen, William J. McBride, Christopher A. D'Souza, Peter Laverman, Charles J. Smith, David M. Goldenberg, Wim J.G. Oyen and Otto C. Boerman

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