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GRPR-selective PET imaging of prostate cancer using [^{18}F]-lanthionine-bombesin analogs

G. Carlucci[a,b], A. Kuipers[c], H.J.K. Ananias[a], D. de Paula Faria[b], R.A.J.O. Dierckx[b], W. Helfrich[d], R. Rink[c], G.N. Moll[c,e], I.J. de Jong[a], P.H. Elsinga[b,*]

[a] Department of Urology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[b] Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[c] Lanthio Pharma, Groningen, The Netherlands
[d] Surgical Research Laboratory, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[e] Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands

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ABSTRACT

The gastrin-releasing peptide receptor (GRPR) is overexpressed in a variety of human malignancies, including prostate cancer. Bombesin (BBN) is a 14 amino acids peptide that selectively binds to GRPR. In this study, we developed two novel Al^{18}F-labeled lanthionine-stabilized BBN analogs, designated Al^{18}F-NOTA-4,7-lanthionine-BBN and Al^{18}F-NOTA-2,6-lanthionine-BBN, for positron emission tomography (PET) imaging of GRPR expression using xenograft prostate cancer models. (Methyl)lanthionine-stabilized 4,7-lanthionine-BBN and 2,6-lanthionine-BBN analogs were conjugated with a NOTA chelator and radiolabeled with Al^{18}F using the aluminum fluoride strategy. Al^{18}F-NOTA-4,7-lanthionine-BBN and Al^{18}F-NOTA-2,6-lanthionine-BBN was labeled with Al^{18}F with good radiochemical yield and specific activity > 30 GBq/μmol for both radiotracers. The log *D* values measured for Al^{18}F-NOTA-4,7-lanthionine-BBN and Al^{18}F-NOTA-2,6-lanthionine-BBN were -2.14 ± 0.14 and -2.34 ± 0.15, respectively. In athymic nude PC-3 xenografts, at 120 min post injection (p.i.), the uptake of Al^{18}F-NOTA-4,7-lanthionine-BBN and Al^{18}F-NOTA-2,6-lanthionine-BBN in prostate cancer (PC-3) mouse models was 0.82 ± 0.23% ID/g and 1.40 ± 0.81% ID/g, respectively. An excess of unlabeled ε-aminocaproic acid-BBN(7–14) (300-fold) was co-injected to assess GRPR binding specificity. Tumor uptake of Al^{18}F-NOTA-4,7-lanthionine-BBN and Al^{18}F-NOTA-2,6-lanthionine-BBN in PC-3 tumors was evaluated by microPET (μPET) imaging at 30, 60 and 120 min p.i. Blocking studies showed decreased uptake in PC-3 bearing mice. Stabilized 4,7-lanthionine-BBN and 2,6-lanthionine-BBN peptides were rapidly and successfully labeled with ^{18}F. Both tracers may have potential for GRPR-positive tumor imaging.

Introduction

Prostate cancer (PCa) is the third-leading cause of cancer related deaths and the most frequently diagnosed cancer among men in the Western World [1]. Early detection of prostate cancer may lead to an improved cure rate. Although transrectal ultrasound-guided biopsies is the gold standard procedure for histological diagnosis, chances of under- or overstaging due to sampling errors in multifocal disease are still common [2]. Furthermore, transrectal ultrasound-guided biopsies have a suboptimal sensitivity, as they can miss up to 35% of cancers [3,4]. Therefore, a sensitive, specific imaging procedure to detect prostate cancer is needed. Such an imaging technique might also be of use for detection and local staging of prostate cancer, guidance for prostate biopsies, application of intensity-modulated radiotherapy on hot-spots, detection of distant metastases or local recurrence and therapy response monitoring. Nuclear imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) are effective diagnostic tools in oncology and can fully fulfill these purposes [5]. Some radiopharmaceuticals such as ^{11}C-choline or ^{18}F-fluoroacetate have already been employed for prostate cancer detection [6]. However, they have limitations due to the relatively low uptake of choline at low PSA levels, the unselective uptake of choline and acetate in both normal and inflamed prostate and the low sensitivity for small-sized metastases. Therefore it is highly important

* Corresponding author at: Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, Hanzeplein 1, 9713 EZ Groningen, The Netherlands. Tel.: +31 50 361 3247; fax: +31 50 3611697.
E-mail address: p.h.elsinga@umcg.nl (P.H. Elsinga).

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to develop accurate new radiopharmaceuticals that specifically target prostate cancer-associated, overexpressed antigens. Gastrin-releasing peptide receptor (GRPR) has emerged, over the years, as a tumor-associated antigen of particular interest. Different reports have demonstrated that GRPR is overexpressed in a variety of cancers such as lung, colon, gastric, pancreatic, breast and prostate, and that the expression levels of GRPR has prognostic value [7–9]. Bombesin (BBN) is a 14-amino acid peptide which shares sequence homology with GRPR [9]. Several BBN sequences have been developed for GRPR-positive tumor-targeted imaging with PET and SPECT [10–14]. Mainly two types of bombesin sequences have been reported, full-length BBN and truncated sequences. Full-length BBN sequences can be easily modified and truncated. Alternatively, synthetic sequences with one or more substituted and/or deleted amino acids can be easily obtained. The major limitation of full-length BBN is its poor in vivo stability. The aim of this study is to evaluate two full-length lanthionine-stabilized BBN analogs (named 4,7-lanthionine-BBN and 2,6-lanthionine-BBN) with respect to their ability to target GRPR and to their increased stability. Lanthionines are thioether crosslinked amino acids that can confer resistance to peptidases even in peptidase-rich homogenates of kidney cortex, liver and pancreas [15–17]. The thioether bridges in lanthionines are much more stable than disulphide bridges and even more stable than peptide bonds [18]. The selected sequences were radiolabeled by ^{18}F via the aluminium ^{18}F-fluoride ($Al^{18}F$) one-pot method pioneered by McBride and co-workers [19–21]. NOTA was used as chelator because of its properties to stabilize the +2 charge of the $Al^{18}F^{2+}$ complex. Here, we describe the radiosynthesis and subsequently the in vitro and in vivo targeting characteristics of the two different lanthionine-stabilized BBN peptides labeled by ^{18}F.

Methods

Reagents and materials

All reagents were purchased from Sigma-Aldrich Chemical (St. Louis, Missouri, USA), and were used as received without further purification. NOTA-NHS was purchased from CheMatech (Dijon, France) and used as received without further purification. Aqueous ^{18}F-fluoride was produced by irradiation of ^{18}O-water with a Scanditronix MC-17 cyclotron via the $^{18}O(p,n)^{18}F$ nuclear reaction. The ^{18}F-fluoride solution was passed through a SepPak® Light Accell plus QMA anion exchange cartridge (Waters, Milford, MA, USA) to recover the ^{18}O-enriched water. C18 cartridges with 55–105 μm particle size were purchased from Waters Corporation (Milford, MA, USA) and were pre-treated with water and acetonitrile before use. ^{125}I-Tyr^4-BBN was purchased from PerkinElmer (Boston, MA). 4-D-Cysteine, 7-L-cysteine BBN and 2-D-cysteine,6-L-cysteine-BBN peptides were purchased from JPT Peptide Technologies (Berlin, Germany) and peptide purity was over 95%. Radioactivity was detected by a Bicron Frisk-Tech area monitor (Umass Lowell, MA). The GRPR-positive human prostate cancer cell line PC-3 (ATCC, Manassas, VA, USA) was cultured in RPMI 1640 medium (Lonza, Verviers, France) supplemented with 10% fetal calf serum (Thermo Fisher Scientific Inc., Logan, UT) at 37 °C in a humidified 5% CO_2 atmosphere. Male athymic nude mice (6 weeks of age, 18–20 g) were purchased from Harlan (Zeist, The Netherlands). Reversed phase high-performance liquid chromatography (RP-HPLC) was performed on a HITACHI L-2130 HPLC system (Hitachi High Technologies America Inc., Pleasanton, CA) equipped with a Bicron Frisk-Tech area monitor (Umass Lowell, MA). Purification of radiolabeled peptides was performed using a reversed phase Alltima Alltech RP-C18 column (10 mm × 250 mm, 5 μm) (Delta Technical Products, Des Plaines, IL). The flow was set at 2.5 mL/min using a gradient system starting from 90% solvent A (0.01 M phosphate buffer, pH = 6.0) and 10% solvent B (acetonitrile) (5 min), followed by a linear gradient mobile phase going to 35% solvent A and 65% solvent B at 35 min and then back to 90% solvent A and 10% solvent B at 40 min (Method A). Quality control was performed using a reversed phase Grace Smart RP-C18 column (Grace, Lokeren, Belgium) (4.6 mm × 250 mm, 5 μm). The flow was set at 1 mL/min using a gradient system starting from 90% solvent A (0.01 M phosphate buffer, pH = 6.0) and 10% solvent B (acetonitrile) (2 min) to 35% solvent A and 65% solvent B at 32 min (Method B). Lyophilization and sample concentration was done in a Speedvac concentrator (Thermo Fisher Scientific Inc., Logan, UT). Mass spectra were recorded with a Matrix-assisted laser desorption/ionization (MALDI) equipped with Time-of-Flight (ToF) (Bruker Daltonics Inc., Billerica, MA). All radioactive counting measurements were obtained on a Compugamma CS1282 (LKB-Wallac, Turku, Finland). All PET imaging experiments were conducted on a microPET INVEON camera equipped with a CT scanner (Siemens, Knoxville, TN) and images reconstructed using INVEON Acquisition Workplace software (Siemens Inveon Software, Erlangen, Germany). Homogenization of organs for stability studies were conducted using an IKA Ultra-Turrax T8 (IKA-Werke GmbH & Co. KG, Staufen, Germany).

Synthesis of stabilized BBN analogs

A small library of lanthionine bombesin variants with lanthionine rings of three or four amino acids considering a lanthionine (Ala-S-Ala) as one amino acid (thioether crosslinks of positions i, i+3 or i, i+4), throughout the bombesin peptide was designed followed by enzymatic and chemical synthesis as described below. Enzymatic synthesis depended on the substrate specificity of the lanthionine-installing enzymes [22,23]. Lanthionine-stabilized peptides with the lanthionine in the C-terminal half could be produced stereospecifically by a three-step method. Lanthionine was firstly introduced via a *Lactococcus lactis* production system [24,25] followed by pGlu formation and finally amidation [26].

Nonspecific base-assisted sulfur extrusion was used for obtaining lanthionine-stabilized peptides with the lanthionines in the N-terminal half [22–24]. Briefly, D-Cys, L-Cys bombesin mutants were dissolved in water [2 mg/mL]. Ammonia was added to a 0.3% final concentration and the reaction mixture incubated at 37 °C overnight. After that, ammonia was removed, the sample was concentrated and lanthionine-bombesin was purified by HPLC. The final isomeric mixtures of the lanthionine bombesin analogs were analyzed by mass spectrometry. Introduction of a lanthionine by desulphurization, causes a loss in mass of 34 Da. We hypothesized that limited reduction in receptor affinity could be more than compensated by increased stability. Two variants were eventually selected on the basis of successful synthesis and receptor affinity. These selected peptides, 4,7-lanthionine-bombesin and 2,6-lanthionine-bombesin, are shown in Fig. 1.

Synthesis of NOTA-4,7-lanthionine-BBN and NOTA-2,6-lanthionine-BBN

NOTA-NHS (50 μmol) and 4,7-lanthionine-BBN (2.6 μmol) or 2,6-lanthionine-BBN (2.6 μmol) were dissolved in 1.5 mL of dimethylformamide (DMF). After addition of excess triethylamine (3 equivalents, 7.8 μmol), the reaction mixture was stirred overnight at room temperature to make sure the reaction was complete. The product was purified by HPLC (HPLC method 1) and collected at 26 min. Lyophilization of the collected fraction gave the final product (~24%) with >95% purity by HPLC. For NOTA-4,7-lanthionine-BBN