Synthesis and positron emission tomography evaluation of ^{18}F-Glu-Urea-Lys, a prostate-specific membrane antigen-based imaging agent for prostate cancer

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Abstract. In recent years, single-photon emission computed tomography and positron emission tomography (PET) have also been used, in addition to computed tomography and magnetic resonance imaging, in targeting the diagnosis of prostate cancer. The aim of this study was to synthesize the prostate-specific membrane antigen (PSMA)-based imaging agent 2-{3-[1-Carboxy-5-(4-[^{18}F] fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid (^{18}F-Glu-Urea-Lys, [^{18}F]3) and to detect its PET imaging efficiency for high PSMA expression in prostate cancer. In this study, ^{18}F-Glu-Urea-Lys was synthesized in two steps from the *p*-methoxybenzyl-protected Glu-Urea-Lys precursor using *N*-Hydroxysuccinimidyl-4-[^{18}F] fluorobenzoate ([^{18}F]SFB). PET imaging evaluation was conducted in nude mice using LNCaP (PSMA$^+$), and PC-3, 231 and A549 (all PSMA$^-$) xenograft models. The results indicated that ^{18}F-Glu-Urea-Lys was produced in radiochemical yields of 28.7%. The radiochemical purity was 99.1% and the mean total synthesis time was 168 min. In nude mice models ^{18}F-Glu-Urea-Lys clearly delineated PSMA$^+$ LNCaP prostate tumor xenografts on PET imaging. At 4 h post-injection, the contrast agents were only observed in renal, liver, bladder and PSMA$^+$ LNCaP tumors. The PSMA$^-$ tumor (PC-3, 231 and A549) was clear. In conclusion, ^{18}F-Glu-Urea-Lys was found to be easily synthesized. This radiotracer demonstrated high tumor and low-to-normal tissue uptake, fast clearance from non-target tissues and retention in PSMA$^+$ prostate tumor xenografts.

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Introduction

Prostate cancer is one of the most common types of tumor and the second highest cause of cancer-related mortality in males (1). The majority of patients succumb to tumor recurrence and metastasis. Early diagnosis, targeted therapy and effective monitoring following radical prostatectomy may have a significant impact on the prognosis of patients. The location of the tumor determines the subsequent treatment. In recent years not only have computed tomography (CT) and magnetic resonance imaging been used in prostate cancer diagnosis, single-photon emission computed tomography (SPECT) and positron emission tomography (PET) also offer new ways of targeting diagnosis (2,3).

Prostate-specific membrane antigen (PSMA) is a type 2 transmembrane glycoprotein expressed in prostate epithelial cells. It is shown to be highly expressed in prostate cancer in a disease progression-dependent manner (4). This study introduces a means of synthesis of 2-{3-[1-Carboxy-5-(4-[^{18}F] fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid (^{18}F-Glu-Urea-Lys, [^{18}F]3). This low molecular weight agent is easily prepared and demonstrates a high uptake in PSMA$^+$ tumors.

Materials and methods

General procedures. All reagents and solvents were purchased from Sigma-Aldrich (Milwaukee, WI, USA). 1H NMR spectra were obtained on an Avance 400 MHz spectrometer (Bruker Corporation, Ettlingen, Germany). Electrospray ionization (ESI) mass spectra were obtained on a Bruker Esquire 3000 plus system. High-performance liquid chromatography (HPLC) purification was performed on a Waters 2998 and Waters 2487 system (Waters Corp., Milford, MA, USA). [^{18}F]-fluoride was obtained using the M-7 Cyclotron (Sumitomo Heavy Industries, Ltd., Tokyo, Japan). Solid-phase extraction cartridges (Sep-Pak C18 Plus) were purchased from Waters Corp. The precursor 2-[3-(5-amino-1-carboxy-pentyl)- ureido]-pentanedioic acid 1 was synthesized in Dalian Medical University, China (5).

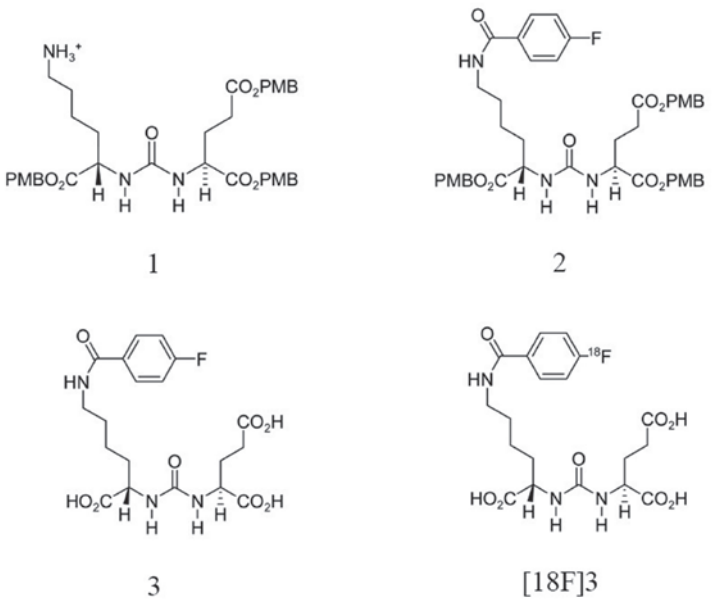

Figure 1. Synthesis process of 2-{3-[1-Carboxy-5-(4-[^{18}F] fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid (^{18}F-Glu-Urea-Lys, [^{18}F]3). Compound 1 reacted with *N*-Succinimidyl 4-fluorobenzoate (SFB) to obtain compound 2. Then the *p*-methoxybenzyl (PMB) protection was removed to obtain compound 3, [^{18}F]3. [^{18}F]3 was synthesized in two steps from compound 1 using *N*-Hydroxysuccinimidyl-4-[^{18}F] fluorobenzoate ([^{18}F]SFB).

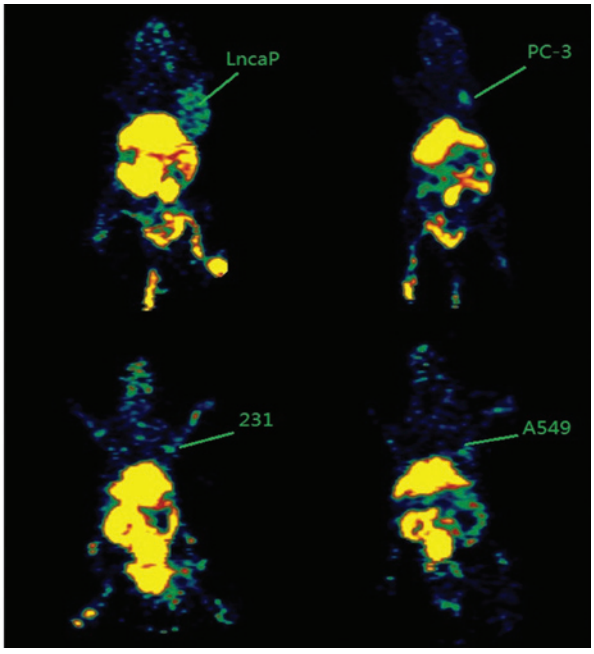

Figure 2. Positron emission tomography imaging. At 4 h post-injection, contrast agents were only observed in renal, liver, bladder and prostate-specific membrane antigen-positive (PSMA$^+$) LNCaP tumors. Almost no radiotracer was observed in PSMA$^-$ tumors (PC-3, 231 and A549).

This study was approved by the ethics committee of the First Affiliated Hospital of Dalian Medical University (Dalian, China).

Cell lines. LNCaP, PC-3, 231 and A549 cells were obtained from WUXI Molecular Imaging CRO (Wuxi, China). Nude mice were purchased from JiangNan University, China. Cells ($5x10^6$) were implanted subcutaneously into the right flank of models. Mice were imaged when the tumor xenografts reached 5-8 mm in diameter.

2-{3-[1-tert-Butoxycarbonyl-5-(4-fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid di-tert-butyl ester 2. *N*-Hydroxysuccinimidyl-4-[^{18}F] fluorobenzoate (SFB, 145 mg), hydroxybenzotriazole (HOBt, 165 mg) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 235 mg) were added to a solution of CH2Cl2 (50 ml), then mixed with Triethylamine (208 mg) and stirred for 1 h. Precursor 1 (500 mg) was added and stirred at room temperature overnight. The crude material was purified on a silica column to obtain 550 mg compound 2.

2-{3-[1-Carboxy-5-(4-fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid 3. Compound 2 (110 mg) was added to HCl/diethyl ether solution (20 ml), and stirred overnight at room temperature. The crude material was purified using HPLC to obtain 24 mg compound 3.

2-[3-[1-Carboxy-5-(4-[^{18}F]fluoro-benzoylamino)-pentyl]-ureido]-pentanedioic acid [^{18}F]3. Compound 1 (1 mg) was added to phosphate-buffered saline (PBS) solution (100 μl). Then [^{18}F]SFB (100 μl) and Na_2CO_3 (40 μl) was added, and the mixture was regulated to pH 7.6, stirred and reacted in an oil bath at 50˚C for 30 min. When the reaction cooled down, trifluoroacetic acid (100 μl) and benzaldehyde (3 μl) were added, and reacted in an oil bath at 50˚C for 30 min. Water (5 ml) was added, then the mixture was purified on a silica column, and washed with acetonitrile (0.5 ml). Finally, it was purified using HPLC to obtain [^{18}F]3.

PET imaging. Small animal PET was used to image the nude mice implanted with PSMA+ (LNCaP) and PSMA- (PC-3, 231 and A549) xenografts. The nude mice were anesthetized with diethyl ether and injected intravenously with 0.2 mCi ^{18}F-Glu-Urea-Lys in 200 μl PBS. The images were obtained at post-injection times of 1, 2 and 4 h.

Results

Synthesis of the compounds 2, 3 and [^{18}F]3. The final quantity of 2-{3-[1-tert-Butoxycarbonyl-5-(4-fluoro-benzoylamino)-pentyl]-ureido}-pentanedioic acid di-tert-butyl ester 2 obtained was 550 mg, with a produce yield of 88%. The associated parameters are listed as the followings: ^{1}H NMR (400 MHz, CDCl3) δ7.91-7.96 (m, 2H), 7.26-7.45 (m, 1H), 7.05-7.11 (m, 2H), 5.70-5.72 (m, 1H), 5.40-5.43 (m, 1H), 4.20-4.23 (m, 2H), 3.34-3.51 (m, 2H), 2.24-2.29 (m, 2H), 2.16 (m, 1H), 1.99-2.04 (m, 2H), 1.64-1.77 (m, 32H). The $[M+H]^+$ ESI mass calculated for $C_{31}H_{48}FN_3O_8$ was 609.7.

The final quantity of 2-{3-[1-Carboxy-5-(4-fluoro-benzoylamino)-pentyl]ureido}-pentanedioic acid 3 obtained was 24 mg, with a produce yield of ~30%. The associated parameters are listed as the followings: ^{1}H NMR (400 MHz, CDCl3) δ8.51 (s, 1H), 7.89-7.92 (m, 2H), 7.27-7.31 (m, 2H), 6.34 (m, 2H), 4.06-4.08 (m, 2H), 3.23-3.55 (m, 3H), 2.25-2.51 (m, 2H), 1.50-1.60 (m, 7H), 1.06-1.35 (m, 3H). The $[M+H]^+$ ESI mass calculated for $C_{19}H_{24}FN_3O_8$ was 441.4.

The radiochemical yield of [^{18}F]3 achieved was 28.7%. The radiochemical purity was 99.1% and the mean synthesis time was 168 min (Fig. 1).

PET imaging. Following the injection, ^{18}F-Glu-Urea-Lys rapidly and notably delineated PSMA$^+$ LNCaP prostate tumor xenografts on the PET imaging. At 4 h post-injection, the contrast was only observed in renal, liver, bladder (the intense renal uptake was partially due to the specific binding of ^{18}F-Glu-Urea-Lys to proximal renal tubules (6) as well as to the excretion of this hydrophilic compound) and PSMA$^+$ LNCaP tumors. PSMA$^-$ tumors (PC-3, 231 and A549) were clear according to the radiotracer (Fig. 2).

Discussion

Due to the relatively low metabolic rate of prostate cancer, PET with [^{18}F] fluorodeoxy glucose (FDG PET) has proven ineffective. Other agents for imaging prostate cancer include the choline series (7), radiolabeled acetates (8), [^{18}F] F-FACBC (9), [^{18}F] FMAU (10) and [^{18}F] FDHT (11). However, each has disadvantages, including cost, difficulty to synthesize or low specificity to prostate cancer.

Overexpressed in prostate cancer, PSMA is becoming an attractive target for cancer imaging and therapy (12). PSMA has an internalization signal that allows internalization of the protein on the cell surface into an endosomal compartment (13). Previous studies reveal that a type of monoclonal antibody against PSMA is available for imaging diagnosis and therapy of prostate cancer (14,15). These agents have long circulation times, low specificity to target tissue and were expensive to synthesize, limiting their clinical use in the diagnosis of prostate cancer.

Maresca *et al* (16) designed and synthesized a type of Glu-Urea-R compound which could be marked by 123I and 131I. This R-group and the substrate coupling with it may notably affect the affinity of the compounds to PSMA. To improve the diagnosis and therapy of prostate cancer, in recent years researchers have developed a series of PSMA-based small molecular agents. This type of agent was based on various R-groups, including [^{11}C] DCMC (17), [^{125}I] DCIT (18) and [^{18}F] DCFBC (19), each having its own benefits.

The use of these compounds is not limited to the area of diagnosis of prostate cancer. Kularatne *et al* (20) coupled the chelate 99mTc-Dap-Asp-Cys with Glu-Urea-R for use in SPECT as an imaging agent. In combination with the chemotherapy drug TubH, this compound was capable of killing PSMA$^+$ LNCaP cells *in vitro*. Zhang *et al* (21) coupled dinitrophenyl (DNP) with Glu-Urea-R to target prostate cancer. The DNP-end increased the immune antibodies and killed the cancer cells.

These small molecular agents demonstrate high specificity and affinity with PSMA (22). The use of ^{18}F-Glu-Urea-Lys provides a new strategy in diagnosis, preoperative or tumor recurrence staging, and also could be extended from molecular imaging to the gene target therapy area.

In conclusion, ^{18}F-Glu-Urea-Lys demonstrated high PSMA$^+$ tumor uptake and low-to-normal tissue uptake. This radiotracer could be quickly cleared from non-target tissues and retention may occur in PSMA$^+$ prostate tumor. With its relatively simple and convenient method of synthesis, this type of PSMA-based small molecular imaging agent may have a variety of clinical uses to help localize prostate cancer.

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