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-\[1-Carboxy-5-(4-[18F] fluoro-benzoylamino)-pentyl\]-ureido\}-pentanedioic acid (\[^{18}\text{F}]\text{Glu-Urea-Lys}, \[^{18}\text{F}]3) and to detect its PET imaging efficiency for high PSMA expression in prostate cancer. In this study, \[^{18}\text{F}]\text{Glu-Urea-Lys} was synthesized in two steps from the \(p\)-methoxybenzyl-protected Glu-Urea-Lys precursor using \(N\)-Hydroxysuccinimidyl-4-[\(^{18}\text{F}\)fluorobenzoate (\[^{18}\text{F}\]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}\text{F}]\text{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}\text{F}]\text{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\(^+\) tumors. The PSMA\(^-\) tumor (PC-3, 231 and A549) was clear. In conclusion, \[^{18}\text{F}]\text{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.

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-\[1-Carboxy-5-(4-[18F] fluoro-benzoylamino)-pentyl\]-ureido\}-pentanedioic acid (\[^{18}\text{F}]\text{Glu-Urea-Lys}, \[^{18}\text{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). \([18\text{F}]\)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).
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 (5x10⁶) were implanted subcutaneously into the right flank of models. Mice were imaged when the tumor xenografts reached 5-8 mm in diameter.

**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 ¹⁸F-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 [18F]3. The final quantity of 2-{3-[1-tert-Butoxycarbonyl-5-(4-fluoro-benzoylelamino)-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: 1H NMR (400 MHz, CDCl3) δ 7.91-7.96 (m, 2H), 7.26-7.45 (m, 1H), 7.05-7.11 (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, 3H). The [M+H]+ ESI mass calculated for C_{24}H_{36}FN_{10}O_{8} was 609.7.

The final quantity of 2-{3-[1-Carboxy-5-(4-fluoro-benzyloxy)-pentyl]ureido}-pentanedioic acid 3 obtained was 24 mg, with a produce yield of ~30%. The associated parameters are listed as the followings: 1H NMR (400 MHz, CDCl3) δ 8.51 (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_{3}O_{7} was 441.4.

PET imaging. Following the injection, [18F]-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 tumor xenografts on the PET imaging). At 4 h post-injection, the contrast was only observed in renal, liver, bladder (the tumor xenografts on the PET imaging). At 4 h post-injection, the contrast was only observed in renal, liver, bladder (the tumor xenografts on the PET imaging). At 4 h post-injection, the contrast was only observed in renal, liver, bladder (the tumor xenografts on the PET imaging).

Discussion

Due to the relatively low metabolic rate of prostate cancer, PET with [18F] fluorodeoxy glucose (FDG PET) has proven ineffective. Other agents for imaging prostate cancer include the choline series (7), radiolabeled acetates (8), [18F] F-FACBC (9), [18F] FMAU (10) and [18F] 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, and affinity with PSMA (16). The use of these compounds is not limited to the area of diagnosis of prostate cancer. Kularatne et al (16) 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 [18F]-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, [18F]-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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References


