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Validation of a [Al^{18}F]PSMA-11 preparation for clinical applications

Ehab Al-Momani*, Ina Israel, Samuel Samnick

Department of Nuclear Medicine, University of Würzburg, D-97080 Würzburg, Germany

HIGHLIGHTS

- An efficient method for preparation of [Al^{18}F]PSMA-11 was developed and validated for nuclear medicine applications.
- [Al^{18}F]PSMA-11 was stable in 1% EtOH/saline as sterile and non-pyrogenic injection solution ready for clinical applications.
- [Al^{18}F]PSMA-11 exhibited higher uptake and retention in PMSA-expressing LNCap prostate cells compared to its ^{68}Ga analogues.

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ABSTRACT

Imaging prostate-specific membrane antigen (PSMA) using positron emission tomography (PET) has been presented so far as the most sensitive and specific with regard to prostate cancer detection, in particular in high-risk prostate cancer patients. Currently, it mainly features Gallium-68 (^{68}Ga) labeled PSMA ligands, notably [^{68}Ga]Glu-urea-Lys(Ahx)-HBED-CC ([^{68}Ga]-PSMA-11) and [^{68}Ga]DOTAGA-FFK (Sub-KuE termed ([^{68}Ga]PSMA-I & T). However, ^{68}Ga has several shortcomings as radionuclide including a short half-life and non-ideal energies. This has motivated consideration of ^{18}F-labeled analogues for PET imaging of prostate cancer.

Here, we describe a simple synthesis and validation of a fluorine-18 labeled Glu-urea-Lys(Ahx)-HBED-CC ([Al^{18}F]PSMA-11) for nuclear medicine applications. An efficient method for preparation of [Al^{18}F]PSMA-11 was developed and validated (according to Pharm Eur) for routinely clinical applications. [Al^{18}F]PSMA-11 was reproducibly obtained in radiochemical yields of 84 ± 6% (n = 15) and > 98% radiochemical purity using an improved one-step radiofluorination in aqueous solution. The total (production/preparation) time, including purification, pharmacological formulation of the isolated product and the quality control of the injectable solution was less than 60 min. The [Al^{18}F]PSMA-11 was stable over 4 h in 1% EtOH/saline selected as injection solution. The solution was sterile, non-pyrogenic and ready for clinical applications after sterile filtration through a 0.22 μm membrane filter under sterile conditions. In addition, [Al^{18}F]PSMA-11 exhibited higher uptake and retention in PMSA-expressing LNCap prostate cells as compared to its clinically established ^{68}Ga-labeled analogues [^{68}Ga]PSMA-11 and [^{68}Ga]PSMA-I & T as well as to [^{68}Ga]NOTA-Bn-PSMA. The simple and fast preparation of [Al^{18}F]PSMA-11 combined with its favorable pharmacological properties warrant its translation to a clinical setting.

Conclusion: The facile and high-yielding radiosynthesis of [Al^{18}F]PSMA-11as well as its promising in vitro and in-vivo characteristics makes it worthy of clinical development for PET imaging of prostate cancer.

1. Introduction

The early detection of prostate cancer (PCa) recurrences and metastases has gained highest clinical impact in molecular imaging during the last years. Among the radiotracers for molecular imaging by positron emission tomography (PET), [^{11}C]- and [^{18}F]-labeled choline derivatives, [^{11}C]-acetate, 1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid, and [^{18}F]-labeled bombesin analogues provided promising advances in imaging of PCa (Beer et al., 2011; Nanni et al., 2013; Sah et al., 2015; Yu et al., 2014). However, numerous studies report a limited sensitivity and specificity of these tracers for imaging PCa, especially in patients with low PSA levels (Yu et al., 2014; Cimitan et al., 2006). Therefore, many efforts have been undertaken toward identification of more sensitive tracers to improve imaging of PCa. Among the currently available strategies, targeting of glutamate carboxy peptidase II, also known as prostate-specific membrane antigen (PSMA) represents the most promising strategy for the development of radiotracers for imaging PCa (Kularatne et al., 2010; Zaheer et al.,

* Correspondence to: Department of Nuclear Medicine, University of Würzburg, Oberdürrbacherstraße 6, D-97080 Würzburg, Germany.
E-mail address: almomani_e@ukw.de (E. Al-Momani).

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2009). PSMA is expressed in nearly all prostate cancers at all stages of disease, and especially in poorly differentiated, metastatic, and hormone-refractory carcinomas. Its expression level is about 1000-fold higher compared to the physiologic levels found in normal tissues and organs (Bostwick et al., 1998; Perner et al., 2007; Silver et al., 1997). For all of these reasons PSMA is becoming increasingly recognized as a viable target for imaging and therapy of prostate cancer (Chen et al., 2011; Haberkorn et al., 2016).

Recently, ^{68}Ga-labeled urea-based PSMA inhibitors were developed for clinical applications. Among them, [^{68}Ga]Glu-urea-Lys(Ahx)-HBED-CC (^{68}Ga-PSMA-11) and [^{68}Ga]PSMA-I & T have shown promising results in detecting recurrent PCa and metastases (Eder et al., 2012; Weineisen et al., 2014, 2015; Afshar-Oromieh et al., 2012; Bluemel et al., 2016; Sahlmann et al., 2016) with ^{68}Ga-PSMA-11 being the most widely studied agent, that in addition currently represents the most promising PET tracer for staging prostate cancer as compared to radiolabeled choline (Afshar-Oromieh et al., 2012; Bluemel et al., 2016; Sahlmann et al., 2016; Maurer et al., 2016). On the other hand, there is a need for ^{18}F-labeled PSMA ligands for clinical routinely applications. Radiolabeling of PSMA peptides with ^{18}F represents an attractive alternative to radiometal-based peptides due to favorable physicochemical and nuclear properties of ^{18}F, including a half-life of 109.8 min, allowing radiosyntheses and imaging studies over a few hours as well as a distribution of the ^{18}F-labeled tracers via the satellite concept to hospitals without access to a cyclotron for clinical studies. Unfortunately, the commonly used direct nucleophilic fluorination procedures with ^{18}F are generally not appropriate for peptides and proteins. Therefore, many indirect approaches have been described (Jacobson et al., 2014; Olberg and Hjelstuen, 2010; Schirrmacher et al., 2007). The ^{18}F-fluorinated prosthetic group can then be conjugated to the peptide or protein by many different methods, including oxime formation, acylation, alkylation, maleimide/thiol coupling, and click chemistry, to name a few (Olberg and Hjelstuen, 2010; Schirrmacher et al., 2007). The entire radiofluorination process, followed by purification often is too long ($>$ 3 h) and lead to low radiochemical yields, thus cumbersome for practical use (Olberg and Hjelstuen, 2010; Schirrmacher et al., 2007). This may hinder the development of new targeting agents of medical interest. Therefore, it would be a major advantage to have a simple, rapid method for binding ^{18}F to a variety of peptides and antibodies under mild conditions for PET imaging. Recently, a new method for ^{18}F-labeling has been reported by D'Souza et al. (2011), McBride et al. (2010, 2012), which reported on the direct labeling of chelate-attached-peptide with aluminum-[^{18}F]fluoride ($Al^{18}F^{2+}$) in aqueous solution. Fluorine binds to most metals forming very strong bond such as Al^{3+} in $Al^{18}F^{2+}$ which can form complexes as known in metal binding chelates (Antonny and Chabre, 1992; Li, 2003; Martin, 1996). The aluminum-fluoride bond is highly stable in vivo, and small amounts of AlF complexes are compatible with biological systems (Antonny and Chabre, 1992; Li, 2003). Recently, a synthesis of Al^{18}F-labeled PSMA-11 ([Al^{18}F]PSMA-11) as an analogue to the established [^{68}Ga]PSMA-11 was reported (Malik et al., 2015; Boschi et al., 2016).

Herein, we described an improved radiofluorination method for [Al^{18}F]PSMA-11 for clinical routine applications under GMP conditions and the process was validated for clinical use. In addition, [Al^{18}F]PSMA-11 was compared with its ^{68}Ga-labeled analogue [^{68}Ga]PSMA-11 and with the newly introduced [^{68}Ga]PSMA-I & T as well as with [^{68}Ga]NOTA-Bn-PSMA in term of their accumulation and retention in PCa cell lines. The stability of the new radiofluorinated PSMA ligand [Al^{18}F]PSMA-11 was additionally determined in human serum. Fig. 1 shows the chemical structure of the used PSMA ligand in cell culture.

2. Materials and methods

All chemicals and solvents were from Sigma-Aldrich (Deisenhofen, Germany) and directly used in synthesis without further purification. The PSMA peptides Glu-NH-CO-NH-Lys(Ahx)-HBED-CC (PSMA-11) and PSMA-I & T were purchased from ABX (Radeberg, Germany) and from Scintomics (Fürstenfeldbruck, Germany), respectively. NOTA-Bn-PSMA was developed by our group and now synthesized commercially by Genaxxon Bioscience (Ulm, Germany). The radiolabeling with Ga-68 was performed as described previously (Eder et al., 2012; Weineisen et al., 2014).

[^{18}F]Fluoride was produced on the PETtrace® cyclotron (GE Medical Systems, Uppsala) at the interdisciplinary PET centre of the University of Würzburg. [^{18}F]Fluoride was produced via a ^{18}O(p,n)^{18}F reaction by irradiating 3.0 mL of 95% enriched [^{18}O]water with 16.5 MeV protons. HPLC analysis was carried out on a Scintomics HPLC-system (Scintomics, Fürstenfeldbruck, Germany) equipped with a reversed-phase column (Nucleosil, C18, 250 × 4.6 mm, CS-Chromatographie, Langerwehe, Germany), a UV-detector (220 and 254 nm) and a gamma-detector for radioactivity. GC-2010 (Shimazu, Kyoto, Japan) to identify EtOH concentration and any possibly residual solvents (MeCN, acetone). Solid-phase extraction (SPE) cartridges Sep-Pak light QMA, Sep-Pak Accell plus CM, and Oasis HLB were from Waters (Milford, MA, USA).

Test for sterility of the injection solutions was performed commercially by L & S (L + S AG, Bad Bocklet-Grossenbrach; Germany), based on the pharmacopeia guidelines (Pharm Eur.).

2.1. Preparation of Glu-NH-CO-NH-Lys(Ahx)-[Al^{18}F]HBED-CC ([Al^{18}F]PSMA-11)

[Al^{18}F]PSMA-11 was synthesized according to the previous reported procedure (Malik et al., 2015) with some modifications: [^{18}F]Fluoride was separated from enriched water by SPE using an anion exchange cartridge (Sep-Pak Accell QMA light), washed with 10 mL water then eluted by 500 µL of 0.9% NaCl solution in two fractions. The second fraction (300 µL) was dried under argon flow at 100 °C. After cooling down to room temperature (r.t), 100 µL acetate buffer (0.5 M, pH 4.2) was added to the dried [^{18}F]fluoride and the concentrated fluoride solution was transferred to a solution of PSMA-11 in water (20 µg, 2 µg/µL), $AlCl_3{\cdot}6H_2O$ (3 µL, 0.01 M) and 120 µL ethanol. The reaction mixture was heated at 50 °C for 15 min and cooled to r.t.. After dilution with 2–3 mL of deionized water, the mixture was passed through a HLB 3 cm^3 cartridge (preconditioned with 10 mL of EtOH and 10 mL trace-select water followed by 10 mL air). The HLB cartridge was then rinsed with water (3 × 1 mL), followed by 500 µL of EtOH. The collected product fraction was evaporated and formulated with 1% EtOH/saline solution and passed through a 0.22 µm sterile filter (Millipore, Cork, Ireland) into a sterile vial (IBA, Berlin, Germany) for further evaluations.

2.2. Quality control

Quality control of the injectable solution were assessed after preparation by means of a gradient HPLC (Scintomics, Fürstenfeldbruck, Germany) and thin layer chromatography (TLC). Aliquot of 20 µL of the solution samples was analyzed by HPLC and TLC. The mobile phase for HPLC analysis consisted of solvent mixtures of MeCN/0.1% TFA (A) and water/0.1% TFA (B); 0–10 min: 100% B linear gradient to 100% A; flow rate 0.7 mL/min. TLC analysis was performed on ITLC-SG stripes (Varian, Lake Forest, USA) as stationary phase, using 1 M ammonium acetate/MeOH (1:1) as mobile phase and a TLC-scanner (mini-GITA®, Raytest, Straubenhardt, Germany) for quantification.

2.3. Validation of the injection solutions of [Al^{18}F]PSMA-11 and in-vitro stability

The radiochemical stability of [Al^{18}F]PSMA-11 was tested in different injectable solutions including: phosphate buffered saline (PBS, pH 7.0), 1% EtOH in acetate buffer, 10% EtOH in acetate buffer and 1%