In-House Preparation and Quality Control of Ac-225 Prostate-Specific Membrane Antigen-617 for the Targeted Alpha Therapy of Castration-Resistant Prostate Carcinoma

Abstract

Purpose: Ac-225 labeled with prostate-specific membrane antigen (PSMA-617), a transmembrane glycoprotein which is highly expressed in prostate carcinoma cells, is presently being considered a promising agent of targeted alpha therapy for the treatment of patients suffering from metastatic castration-resistant prostate cancer. In the present study, we report an optimized protocol for the preparation of therapeutic dose of Ac-225 PSMA-617 with high yield and radiochemical purity (RCP).

Methods: Ac-225 PSMA-617 was prepared by adding the peptidic precursor-PSMA-617 (molar ratios, Ac-225: PSMA-617 = 30:1) in 1 ml ascorbate buffer to Ac-225 and heating the reaction mixture at 90°C for 25 min to obtain the radiopptide with high RCP and yield. The radiolabeled peptide was administered in patients who met the eligibility criteria and posttherapy assessment was done.

Results: Ten batches of Ac-225 PSMA-617 were prepared following this protocol. The radiopptide was obtained with an adequate yield of 85%–87% and RCP of 97%–99%.

Conclusion: The current protocol allows single-step, successful, routine inhouse radiolabeling of Ac-225 with PSMA-617 with high yield and RCP.

Keywords: Ac-225 prostate-specific membrane antigen-617, labeling, prostate cancer; radiochemical purity, targeted alpha therapy

Introduction

Prostate cancer (PC) is the second most common cause of cancer in men of all ages.\(^1\) Prostate-specific membrane antigen (PSMA) is a 750 amino acid type II transmembrane glycoprotein which is highly expressed in prostate carcinoma cells. After binding at the tumor cell surface, the PSMA ligands are internalized allowing radioisotopes to be concentrated within the cell and therefore, a suitable target for PC imaging and therapy.\(^2\) An increasingly popular alternative can be the use of \(\alpha\)-emitting radionuclides for the therapy of PC patients. Till date, promising preclinical and clinical results have been reported for a number of \(\alpha\)-emitters, such as Bi-213, Ac-225, and Ra-223 which have successfully been used to treat different tumors.\(^3\)–\(^5\) Alpha (\(\alpha\))-particle is an ionized\(^4\) He nucleus with a +2 electric charge and therefore results in more effective ionization with and high linear energy transfer of the order of magnitude of 100 keV/\(\mu\)m, over a short range (100 keV/\(\mu\)m, usually of 50–100 \(\mu\)m in a soft tissue), typically just a few cell diameters.\(^6\) This high linear energy transfer may allow for an accurately controlled therapeutic modality that can be targeted to selected malignant cells with a negligible comparably low burden to normal tissues. These attributes combine to provide the rationale for using alpha-particle-emitting radionuclides for cancer therapy.\(^7\) Lately, Ac-225 is being considered as a favorable isotope for TAT due to its 9.9-day half-life allowing enough time for accumulation at the tumor site and the emission of four alpha particles in its decay chain (it decays to stable Bi-209 via four alpha- and two beta-decays) which results in a larger dose to the tumor site. The use of Ac-225 PSMA-617 (Ac-225 PSMA) for the treatment of metastatic castration-resistant PC shows some particularly promising results and its recent commercial availability in high purity has rendered it one of the most promising targeted alpha therapy agents.\(^8\)–\(^9\) However, despite promising results from recent clinical trials, Ac-225 radiopharmaceutical...
development still faces significant challenges that must be overcome to realize the widespread clinical use of Ac-225. Some of these challenges include the limited availability of the alpha emitter, the challenging chemistry required to isolate Ac-225 from any simultaneously produced isotopes, and the need for stable targeting systems with high radiolabeling yields. One of the reasons for restrained actinium chemistry has been the lack of any stable actinium isotopes and as a consequence of which, the chemistry of Ac (III) is virtually unknown. Despite the unique coordination preferences of the large +3 actinide, very few studies investigating new ligands specifically designed to coordinate Ac (III) could be found throughout the literature. The majority of initial Ac-225 chelation studies have focused on screening a variety of commercially available polycyclic or acyclic ligands for their ability to bind Ac-225 and form stable complexes in vitro or in vivo.

We, in our department, have started the in-house synthesis of Ac-225 PSMA for the therapy of patients of metastatic castration-resistant PC. The current study reports an optimized protocol for the radiosynthesis and quality control of the therapeutic dose of Ac-225 PSMA in routine clinical practice.

Materials and Methods

Chemicals and general laboratory

Ultrapure water (Invitrogen, UPTM Distilled water) was used for all the reagents and buffer solutions. Ascorbic acid (JT Baker), Sodium ascorbate (Caelo), Hydrochloric acid Suprapur, (30%), and Instant thin-layer chromatography-Silica gel (ITLC-SG) paper were procured from Merck. Sep-Pak C18 light cartridges were purchased from Waters. Syringe filters (0.22 µm pore size) were purchased from Millipore. Other chemicals such as tri-Sodium citrate dehydrate (Emplura®) and Diethyltriamine pentaacetic acid (Board of Radiation and Isotope Technology) were all of analytical grade. All radioactivity measurements were done using Capintec CRC® 25 dose calibrator with a calibration factor (#775) and multiplying the displayed value with 5 to get the amount of Ac-225 activity.

PSMA-617 in lyophilized bulk 0.5 mg or 1 mg (ABX, GmbH) was reconstituted in the ultrapure water. The reconstituted peptide was stored in a deep freezer at −20°C. Noncarrier added 225Ac chloride free of Actinium-227 (ITG GmbH) dissolved in 0.1 M Hydrogen chloride acid was obtained in a 2 ml vial (specific activity >540 MCi/mg, radionuclidic purity >99%). Sodium ascorbate buffer (pH = 7.5) was prepared by dissolving 40 mg of ascorbic acid with 160 mg of sodium ascorbate in 1 ml of ultrapure water. 0.1 M sodium citrate was prepared by dissolving 1.47 g of trisodium citrate in 50 mL of ultrapure water and was used as the mobile phase for ITLC.

Radiolabeling of the PSMA-617 to 225Ac

Ac-225 PSMA was prepared by adding the required amount (50–150 µg) of the peptidic precursor (PSMA-617) in 1 ml ascorbate buffer. The different ligand-to-metal ratio was investigated in four batches to find the most suitable metal: ligand ratio (M: L). The resulting solution was slowly added to Ac-225 solution vial (2000 µL), gently shaken, and incubated at 90°C for 25 min (started in advance to have a stable temperature). By the time, the light C18 cartridge required for purification was preconditioned by 2 mL of ethanol followed by air and then rinsed with 10 mL of ultrapure water followed by air to dryness.

After cooling at room temperature, the reaction mixture was passed through a preconditioned light C18 cartridge to release free Ac-225 radionuclide. The vial was then washed with 5 mL water and purged with air to dryness and the effluent was collected in a waste vial. The radiolabeled PSMA stored in the C18 cartridge was eluted with 1 ml of 70% ethanol. The cartridge was purged with 5 mL of 0.9% saline solution and air to dryness. An aliquot of the reaction mixture was withdrawn for quality control procedures, as discussed in the next section. After the quality control studies, 15 mg of diethylenetriaminepentaacetic acid (DTPA) reconstituted in 1 ml saline was added to the reaction mixture to scavenge free radionuclides. The preparation was subjected to Millipore filtration prior to the administration to the patients and was delivered as 10 ml solution with the saline/DTPA-mixed solution.

Physicochemical quality control

Before the product was administered to the patients, quality control tests were performed.

Visual appearance

The product was visually checked and pH was determined using the pH paper.

Radiochemical purity

Radiochemical purity (RCP) of Ac-225 PSMA was determined by ITLC as fast as possible after the radiopharmaceutical purification and formulation to prevent false low-yield result caused by free decay products in the Ac-225 chain for each batch of synthesis. Sodium citrate buffer (0.1 M) was used as mobile phase and silica gel impregnated aluminum strips (ITLC-SG strips) were used as a stationary phase. A volume of 3–4 µl drop of sample solution was spotted about 1 cm from one end of the strip and the strip was developed in the sodium citrate buffer till the solvent migrated to the top. The developed TLC strip was read in the TLC scanner (Lablogic) to determine the RCP using a ZnS detector.

Stability studies

Ac-225 PSMA was stored at room temperature and its stability was estimated by performing iTLC at regular time.
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Intervals using ITLC strips and 0.1M sodium citrate as stationary phase and mobile phase, respectively, as already mentioned. The chromatography strip, after development was stored for at least 1 h until radiochemical equilibrium was obtained between Ac-225 (half-life [T½], 9.9 d) and its daughter nuclide Fr-221 (T½, 4.8 min). The activity of the 218-keV γ-emission of Fr-221 was subsequently measured on the upper and lower parts of the strip using high-resolution γ-spectrometry (Ortec) and RCP was determined at the interval of 1 h up to 4 h.

Sterility and apyrogenicity

All the procedures were performed in sterile conditions in a laminar flow to maintain sterility. However, to test the sterility of Ac-225 PSMA-617, the sample solution was incubated in tryptic soya broth at 37°C. Turbidity in the incubated samples was observed for up to 7 days to check the presence of any microorganism. The level of pyrogenicity in the sample was evaluated by the Pyrogen Plus Limulus amebocyte Lysate Kit (Charles River) in accordance with USP XXX1 (Sensitivity 0.125 EU/ml) which is based on the principle of kinetic chromogenic technique. 25 µl of the sample was placed on the PTS™ cartridge reader followed by incubation at 37°C for 15–20 min. This test was performed on a “post hoc” basis.

Patient studies

Eligible patients were men, aged 18 years or older, with histologically confirmed prostate adenocarcinoma. Patients with neuroendocrine differentiation or small cell subtypes were not eligible. Other inclusion criteria included PC progression documented by either rising serum prostate-specific antigen levels or radiological progression; a castrate testosterone concentration of <50 ng/dL; an Eastern Cooperative Oncology Group performance status of 2 or less; and adequate organ function (including haemoglobin ≥9 g/dL, platelet counts >100,000 thou/ml, eGFR >60 ml/min, and albumin >2.5 g/dl). All patients underwent a Ga-68 PSMA-11 scan prior to the therapy. The patients were premedicated with intravenous administration of 8 mg of ondansetron and dexamethasone. Table 1 shows the clinical details of two patients who were treated with Ac-225 PSMA-617 in the department.

Results

Preparation of Ac-225 PSMA

Initially, four batches of Ac-225 PSMA were prepared, the results of which are summarized in Table 2. From the limited number of batches prepared, it was observed that heating the reaction mixture was incubated at 90°C for 25 min and maintaining the metal: ligand ratio of 1:30 lead to obtain the radiopeptide with good high radiochemical yield (RCY) (85%–87%) as well as RCP (97%–99%). Therefore, the corresponding procedure was chosen for regular preparations of 225Ac-PSMA batches for patients [Batch 5-14, Table 2].

Clarity and pH

The final radiolabelled peptide was a clear solution with no particulate matter or milky appearance. The pH of the final product was between 4.5 and 5.5 in all the batches.

Radiochemical purity analysis

According to ITLC findings, Ac-225 PSMA was retained at the point of spotting, whereas free Ac-225 moved to the solvent front as shown in Figure 1. RCP of Ac-225 PSMA was 97%–99.5% for all the batches.

Stability studies

The mean RCP (±SD) of the radiolabeled peptide was 98.8% ± 0.6% up to 3 h which fell to 94.8% at 4 h, as shown in Figure 2.

Sterility and pyrogenicity

The radiolabeled doses were proven to be pyrogen free. Bacterial endotoxin level in all the samples was <0.1 EU/ml. All samples were found to be sterile on incubation for up to 7 days at 37°C, indicating that the samples were suitable for intravenous administration.

Patient studies

A planar whole-body scan was acquired with a gamma camera (Philips Brightview) using a high energy all-purpose collimator at a bed speed of 6 cm/h with a 20% energy window peaked at 440 keV at 24 h which

| Patient number | Age/sex | Clinical details                                                                 | Gleasons score | PSA (ng/ml) | Activity administered (MBq) |
|----------------|---------|--------------------------------------------------------------------------------|----------------|-------------|----------------------------|
| 1              | 65/male | Metastatic PCa, underwent bilateral orchidectomy, radiotherapy and 6 cycles of chemotherapy presented with extensive skeletal metastasis | 8/10           | 56.5        | 6 (162 µCi)                |
| 2              | 69/male | Metastatic PCa, underwent bilateral orchidectomy, radiotherapy and 6 cycles of chemotherapy presented with extensive skeletal metastasis | 9/10           | 150.2       | 7 (189 µCi)                |

PSA: Prostate specific antigen, PCa: Prostate cancer
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Table 2: Details of synthesis of Ac‑225 prostate‑specific membrane antigen‑617

| Batch number | Specific activity of 225Ac (MBq/mg, mCi/mg, nmol) | Activity used MBq, (mCi) | Amount of ligand (μg, nmol) | Ligand:metal ratio | RC yield (%) | RC purity (%) | Apparent molar activity (MBq/µg, mCi/µg) | Amount of peptide (µg) per mCi activity at the end of synthesis |
|--------------|--------------------------------------------------|--------------------------|-----------------------------|-------------------|--------------|--------------|------------------------------------------|--------------------------------------------------------------|
| 1            | 19,980, 540, 0.004                               | 18.5, 0.5                | 50, 0.041                   | 10.23             | 70           | 97.7         | 0.370, 0.01                             | 142.85                                                       |
| 2            | 19,980, 540, 0.004                               | 18.5, 0.5                | 100, 0.082                 | 20.5              | 75           | 98.1         | 0.185, 0.005                            | 266.66                                                       |
| 3            | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 85           | 98.8         | 0.123, 0.003                            | 352.94                                                       |
| 4            | 19,980, 540, 0.004                               | 18.5, 0.5                | 200, 0.164                 | 41                | 87           | 99.1         | 0.0925, 0.002                           | 459.77                                                       |
| 5            | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 86           | 98.8         | 0.123, 0.003                            | 348.83                                                       |
| 6            | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 85           | 98.5         | 0.123, 0.003                            | 352.94                                                       |
| 7            | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 87           | 98.7         | 0.123, 0.003                            | 344.82                                                       |
| 8            | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 86           | 98.8         | 0.123, 0.003                            | 348.83                                                       |
| 9            | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 86           | 99.1         | 0.123, 0.003                            | 348.83                                                       |
| 10           | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 87           | 99.0         | 0.123, 0.003                            | 344.82                                                       |
| 11           | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 87           | 98.8         | 0.123, 0.003                            | 344.82                                                       |
| 12           | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 88           | 98.9         | 0.123, 0.003                            | 340.90                                                       |
| 13           | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 88           | 98.8         | 0.123, 0.003                            | 340.90                                                       |
| 14           | 19,980, 540, 0.004                               | 18.5, 0.5                | 150, 0.123                 | 30.8              | 88           | 99.3         | 0.123, 0.003                            | 340.90                                                       |

RC: Radiochemical

![Figure 1: Typical thin-layer chromatography patterns of Ac-225 prostate-specific membrane antigen-617 complex (retention factor-0.062, radiochemical purity – 98.1%)](image1)

![Figure 2: Radiochemical purity of Ac-225 prostate-specific membrane antigen -617 stored at room temperature for 4 h](image2)

demonstrated the tracer’s distribution corresponding to the uptake in Ga‑68 PSMA‑11 positron‑emission tomography/ computed tomography (PET/CT) scan, as shown in the patient studies. The response to the α‑therapy in patients with PC treated with Ac‑225 PSMA synthesized in the department was assessed with Ga‑68 PSMA‑11 scans at 2 months post therapy. The patient studies have shown near‑complete resolution of skeletal metastasis [Figure 3] and resolution of lung and visceral metastasis [Figure 4] in the posttherapy Ga‑68 PSMA PET/CT scan.

Discussion

PC is one of the leading causes of cancer death in men of all races. Therefore, interest in treatment of PC with PSMA‑targeted α‑radiation therapy agent: Ac‑225 PSMA is increasingly growing all over the world. In consideration of the fact that targeted α‑therapy probably offers various advantages over the use of β‑emitters, our department has recently started the in‑house synthesis of Ac‑225 PSMA radiopharmaceutical drug for the therapy of mCRPC patients.

The current Ac‑225 labeling methods are adequate for clinical trials, but low yields are a problem. The foremost challenge for Ac (III) labeling has been the hunt for a chelating agent that binds Ac (III) with sufficient stability and also controls the release of recoil daughter nuclides. The majority of initial Ac‑225 chelation studies had focused on commercially available polydentate macrocyclic such as 1,4,7,10, tetraazacyclododecane‑1,4,7,10 tetra acetic acid (DOTA), 1,4,7,10‑tetraazacyclododecane‑1,4,7,10‑tetrapropionic acid (DOTPA), and 1,4,7,10‑tetraazacyclododecane‑1, 4,7,10‑tetramethylene‑phosphinic acid (DOTMP) or acyclic ligands such as DTPA and for their ability to
bind Ac-225 and form stable complexes \textit{in vitro} or \textit{in vivo}.\textsuperscript{[10]}

In the study presented by Davis \textit{et al.},\textsuperscript{[11]} ligands PEPA 1,4,7,10,13-pentaazacyclopentadecane-N, N', N'', N''', N'''''-pentaacetic acid, N5O5, ethylenediaminetetraacetic acid, N2O4, and CHX-A"-DTPA (cyclohexyl-DTPA, N3O5) were radiolabeled with Ac-225 with RCY of 80\%–90\% and their data suggested Ac-225 CHX-A"-DTPA to be the most effective tested chelator complex with regard to its \textit{in vivo} stability; however, improvements could still be made to further reduce nontarget tissue accumulation.

McDevitt \textit{et al.}\textsuperscript{[12]} screened the Ac-225 \textit{in vitro} stability and radiolabeling efficiency of several polydentate chelators including DTPA, DOTA, DOTPA (N4O4), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid, N4O4, DOTMP (N4O4), and 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetrapropionic acid, N4O4. According to them, of the six ligands tested, only DOTA and DOTMP showed any complexation of Ac-225 after 2 h at 37°C with RCYs of >99\% and 78\%, respectively. Subsequently, \textit{in vitro} stability assays in serum suggested that the Ac-225 DOTA complex was robust, remaining >90\% intact after 10 days, while the Ac-225 DOTMP complex rapidly dissociated.

Kratochwil \textit{et al.}\textsuperscript{[3]} in their clinical study labeled Ac-225 with PSMA-617 by adding an aliquot of Ac-225 stock solution to a microwave vial containing 0.1 M Tris buffer (pH 9) and an appropriate amount of PSMA-617 stock solution and heated the mixture to 95°C for 5 min using a microwave synthesizer (Biotage Initiator). They obtained a RCP (±SD) of the radiolabeled peptide of 98.8\% ± 0.8\% by ITLC with 0.05 M citric acid (pH 5) as the solvent. Sathege \textit{et al.}\textsuperscript{[9]} used the same method for synthesis and quality control procedures (ITLC) and administered the radiolabeled peptide (Ac-225 PSMA) in patients.

In the current study, Ac-225 was labeled with PSMA-617 in a single-step approach using Na-ascorbate and ascorbic acid and heating the mixture at 90°C for 25 min. Different molar ratios of PSMA-617 and Ac-225 were used to optimize the radiolabelling procedure. It was observed that when the molar ratio of PSMA-617 and Ac-225 was taken as 30:1, the Ac-225 PSMA complex could be obtained with adequate yield (85\%–87\%) and high RCP (97-99\%). Therefore, it was decided that similar molar ratios will be maintained during the preparation of future patient doses of Ac-225 PSMA.

Target specific therapeutic agents need to be prepared with high specific activity to achieve maximum therapeutic
efficacy; therefore, the availability of high specific activity Ac-225 (540 mCi/mg) was a step forward. To ensure that the radiopharmaceutical is prepared with the desired radiocolchemical yield and high RCP, strict quality control procedures were followed before it was administered to the patients. For this, an instant quality control method was developed based on a simple chromatography technique using sodium citrate (0.1 M) solution as the mobile phase and ITLC strips as a stationary phase. This technique provided a fair idea about the suitability of the preparation for human administration and thus can be used as a quick quality control measure. The advantage of the present chromatography system was that a single chromatography system was found sufficient to separate the desirable form from the undesirable species. Furthermore, till date, there is no suitable high-performance liquid chromatography (HPLC) setup for Ac-225 radiopharmaceuticals and the detection of multiple decay modes as well as recovery from the HPLC columns have not been presented in literature. The results of the quality control procedures of the current study were in line with already published studies.[3,9]

Due to the recoil of daughter radionuclides[13] and to prevent any potential risks, the administration of the radiolabelled product was done as soon as possible after the preparation and quality control procedures. However, it was seen that the radiolabeled peptide was stable over 3 hrs. All the samples also passed sterility and endotoxins test indicating the suitability of the radiopharmaceutical for intravenous human administration.

The radiolabeled peptide prepared by this method was administered to the patients of metastatic relapsed cancer prostate at a dose of 100 KBq/kg body weight. The whole-body planar scans done 24 h posttherapy show uptake of 225Ac-PSMA-617 at expected regions. The Ga-68 PSMA scintigraphic scans done at baseline and posttherapy show a good response, as shown in the figures; however, the efficacy and safety of the therapy in these patients are being evaluated and the results will be presented in a separate study.

The advantage of this protocol for in-house preparation of Ac-225 PSMA is that it can allow targeted alpha therapy even in resource-constrained nuclear medicine setups worldwide.

**Conclusion**

PSMA-617 was successfully radiolabeled with Ac-225 with an adequate yield of >85 and RCP of >97%. This protocol has been successfully employed for the single-step routine, in-house synthesis of Ac-225 PSMA for the treatment of patients with metastatic castration-resistant PC.

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**Conflicts of interest**

There are no conflicts of interest.

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