Journal of Pharmaceutical and Biomedical Analysis 163 (2019) 39–44

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Single vial cold kits optimized for preparation of gastrin releasing peptide receptor (GRPR)-radioantagonist ^{68}Ga-RM2 using three different ^{68}Ge/^{68}Ga generators

Kusum Vats, Rohit Sharma, Mythili Kameswaran, Drishty Satpati*, Ashutosh Dash

Radiopharmaceuticals Divisiona, Bhabha Atomic Research Centre, Mumbai, India

ARTICLE INFO

Article history:
Received 24 July 2018
Received in revised form
24 September 2018
Accepted 25 September 2018
Available online 26 September 2018

Keywords:
^{68}Ga-RM2
Cold kits
^{68}Ge/^{68}Ga generators
Gastrin releasing peptide receptor (GRPR)
Prostate cancer

ABSTRACT

^{68}Ga-RM2 is a gastrin releasing peptide receptor (GRPR) antagonist PET (positron emission tomography) radiotracer which is being investigated in clinical trials as a potential prostate cancer imaging agent. Simple, one-step kit formulation of ^{68}Ga-RM2 would facilitate multicentre trials and allow easier integration in hospital radiopharmacy. Herein we report development of three sets of single-vial RM2 cold kits validated for formulation with three respective ^{68}Ge/^{68}Ga generators eluted in 0.6 M, 0.1 M and 0.05 M HCl (hydrochloric acid). Cold kits of varied pH (2, 3, 4 and 5) were prepared using 2 M sodium acetate for three different ^{68}Ge/^{68}Ga generators to determine influence of pH on the radiochemical yield of ^{68}Ga-RM2. Buffer content was optimized with respect to volume of $^{68}GaCl_3$ eluate to be added (1 mL/2 mL/5 mL). Sterility, apyrogenicity and long term stability of cold kits; in vitro and serum stability of ^{68}Ga-RM2 were investigated. In vitro cellular uptake and inhibition studies were performed to demonstrate the specificity of kit-formulated ^{68}Ga-RM2. The radiochemical yield of ^{68}Ga-RM2 formulated from three different generators was observed to be maximum at pH 3 (99 ± 0.5%). Cold kits stored for 6 months at 0 °C also resulted in high radiochemical yield. ^{68}Ga-RM2 exhibited excellent in vitro stability (1 h) and serum stability (1 h). In vitro cellular uptake of 5 ± 0.8% in PC3 cells with >85% inhibition was observed for the ^{68}Ga-RM2 radiotracer indicating its specificity towards GRPR expression. These simple, robust kits shall allow hospitals with different generators to participate in clinical studies of ^{68}Ga-RM2 for screening of GRPR-expressing prostate tumors.

1. Introduction

The worldwide commercial coverage of ^{68}Ge/^{68}Ga generators has revolutionized ^{68}Ga-PET diagnostics [1–4]. The generator technology has ensured feasibility of cyclotron-independent production of PET radiopharmaceuticals at nuclear medicine departments. The global prospects of PET imaging have widened due to extension of ^{68}Ga-radiopharmacy to remote hospitals. ^{68}Ga-labeled peptides targeting receptor over expression at cancer sites have been extensively studied and are at various stages of development [4,5]. The leading ^{68}Ga-radiotracers employed in PET imaging are ^{68}Ga-DOTATATE (somatostatin receptors), ^{68}Ga-PSMA-11 [prostate specific membrane antigen (PSMA)], ^{68}Ga-RM2 [gastrin-releasing peptide receptors (GRPR)], ^{68}Ga-NODAGA-RGD [integrin receptors], ^{68}Ga-DOTA-exendin-4 [glucagon-like peptide 1 receptors (GLP1R)] and ^{68}Ga-pentixafor [chemokine receptor-4 (CXCR4)] [6–11]. Amongst these ^{68}Ga-DOTATATE is now a FDA-approved radiopharmaceutical for neuroendocrine tumor (NET) imaging, ^{68}Ga-PSMA-11 is in phase II/III clinical trials for imaging of prostate carcinoma and ^{68}Ga-RM2 is under phase II study for PET/CT of GRPR-expressing prostate cancers. The favorable pharmacokinetics along with the absence of uptake in salivary/ lacrimal glands has indicated the potential of ^{68}Ga-RM2 as a prospective PET radiotracer for imaging of primary prostate cancer [12]. The kit-type labeling would attract more hospitals to participate and thus expedite multicentre trials of ^{68}Ga-RM2. However unlike the SPECT variant, ^{99m}Tc there are no commercially available 'cold' kits for production of ^{68}Ga-radiopharmaceuticals which is highly desirable for further augmentation of ^{68}Ga-PET. Robust, single vial kits shall allow for rapid and simple radiolabeling procedures that can be easily adapted at hospital radiopharmacy.

Commercial ^{68}Ge/^{68}Ga generators are composed of different column matrix (inorganic/ organic) and are accordingly eluted in varied HCl concentration. The three different generators available

* Corresponding author.
E-mail address: drishtys@barc.gov.in (D. Satpati).

https://doi.org/10.1016/j.jpba.2018.09.045
0731-7085/

are: iThemba Labs, South Africa (SnO_2-based, 0.6 M HCl); Eckert& Ziegler, Germany (TiO_2-based, 0.1 M HCl) and ITG Isotope Technologies, GmbH, Germany (silica matrix, 0.05 M HCl) [1,13]. Elution of $^{68}GaCl_3$ in acidic hydrochloric acid solution in contrast to elution of ^{99m}Tc in neutral sodium chloride solution pose stringent requirement on kit composition. The kit contents also have to be varied depending on the volume of $^{68}GaCl_3$ (in HCl) to be used for preparation of radiolabeled product. The availability of $^{68}GaCl_3$ from three different generators in different HCl molarities would entail development of generator-specific cold kits.

Herein, three sets of RM2 cold kits have been designed for radiolabeling with $^{68}GaCl_3$ eluted directly in 0.6 M, 0.1 M or 0.05 M HCl from three respective SnO_2, TiO_2 and silica-based generator systems available in the market. The effect of buffer content on final pH of the radiolabeling mixture and variation of radiolabeling yield with pH has been investigated. Buffer content was also optimized with respect to volume of $^{68}GaCl_3$ eluate (1 mL/2 mL/ 5 mL). Sterility and long term stability of cold kits, in vitro and serum stability of radiotracer have been examined. In vitro cell studies to determine specificity of kit-formulated ^{68}Ga-RM2 have also been carried out.

2. Materials and methods

2.1. Materials and reagents

Solvents and chemicals were purchased from Aldrich (Milwaukee, WI) unless stated otherwise and used without further purification. 9-Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids, coupling reagents and Rink amide 4-methyl-benzhydrylalanine (MBHA) resin were purchased from NovaBiochem, (Germany). The bifunctional chelator DOTA-tris(tBu)-ester was purchased from ABX advanced biochemical compounds (Biomedizinische Forschungsreagenzien GmbH, Radeberg, Germany). A 30% solution of suprapur® hydrochloric acid (HCl) and sodium acetate were purchased from Fluka Analytical (Steinheim, Germany). Human prostate carcinoma PC3 cells were obtained from the National Center for Cell Sciences (NCCS) Pune, India. Mass spectrum of the peptide was recorded on Advion Mass Spectrometer, USA using electron spray ionization (ESI) in positive mode. High-performance liquid chromatography (HPLC) grade water was obtained from Merck. All other solvents and chemicals were purchased from Sigma Aldrich, USA. All radioactive counting associated with the radiochemical studies were carried out using a well-type NaI(Tl) scintillation gamma counter (Electronic Corporation of India Limited, India). Purification of peptide was carried out using a semi-preparative HPLC system (JASCO, Japan) connected with JASCO-PU-2086 Plus, intelligent prep pump, JASCO UV-2075 Plus absorption detector and having a Megapak Sil C18-10 column (7.5 × 250 mm). The analytical HPLC measurements were performed on a JASCO PU 2080 Plus dual pump HPLC system, Japan, with a JASCO 2075 Plus tunable absorption detector and a Gina Star radiometric detector system, using a C18 reversed phase HiQ Sil column (5 μm, 4 x 250 mm). The eluting solvents (1 mL/min) used in HPLC were; H_2O (solvent A) and acetonitrile (solvent B) with 0.1% trifluoroacetic acid following the gradient: 0–28 min: 90% A-10% A; 28–30 min: 10% A; 30–32 min: 10% A-90% A.

2.2. Peptide synthesis

The peptide RM2 was manually synthesized by standard Fmoc solid phase synthesis on Rink amide MBHA resin [14]. Coupling of each amino acid to build the sequence 4-amino-1-carboxymethylpiperidine-D-Phe-Gln(Trt)-Trp(Boc)-Ala-Val-Gly-His(Trt)-Sta-Leu-NH_2 was carried out using standard Fmoc strategy where 3-fold excess of amino acid as well as O-(7-azabenzotriazol-L-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) was used along with 6-fold excess of DIPEA in DMF (1 mL) for 90 min. The reaction was monitored by picrylsulphonic acid test (20 μL) and Fmoc groups were removed by treatment with 20% piperidine in DMF (1 mL) for 30 min. After assembling the fully protected peptide, 3 eq of DOTA-$(tBu)_3$-ester with 3 eq of HATU and 6 eq of DIPEA was coupled to the N-terminus of the peptide. The resin was then treated with cocktail mixture (1 mL) of TFA/H_2O/TIPS (95:2.5:2.5 v/v/v) for cleavage of the peptide and deprotection of side chain groups. The crude peptide was purified by semi-preparative HPLC and characterized by mass spectroscopy. ESI-MS: *m/z* 1639.0 (obsvd.), 1639.2 (calcd.).

2.3. $^{68}Ge/^{68}Ga$ generators

The radioisotope, ^{68}Ga was eluted from three different generators for standardization of kit components. *Generator A*: SnO_2-based generator eluted in 0.6 M HCl (iThemba Labs, S Africa) (222 MBq, 6 mCi). *Generator B*: TiO_2-based generator eluted in 0.1 M HCl (Eckert & Ziegler, Germany) (222 MBq, 6 mCi). *Generator C*: silica-based generator eluted in 0.05 M HCl (Isotope Technology Garching GmbH, Germany) (370 MBq, 10 mCi). $^{68}GaCl_3$ solution eluted from three generators was utilized for kit formulation without any purification.

2.4. Optimization of radiolabeling conditions

Prior to kit preparation reaction parameters were optimized by addition of 1 mL of $^{68}GaCl_3$ eluted in 0.05 M HCl from generator A. Varied precursor amounts (10, 20, 30, 40 and 50 μg) were tested for radiolabeling with ^{68}Ga at pH 3 followed by heating at 90 °C for 10 min. To determine influence of pH radiolabeling was carried out at pH 2, 3, 4 and 5 using standardized amount of precursor. Temperature variation was performed (27, 50, 75 and 90 °C) followed by heating times experiment (5, 8, 10 and 15 min) at optimized temperature. The radiochemical yield was determined by TLC (acetonitrile/water; 1:1, v/v) and HPLC.

2.5. Kit preparation

Stock solutions of 2 M sodium acetate and RM2 peptide (1 mg/mL) were prepared in sterile distilled water. The optimized peptide amount (40 μg) was used in preparation of all the kits.

Kits for *Generators A, B and C* were prepared with 2 M sodium acetate solution. Varied volumes of sodium acetate solution were added to the kits to obtain final radiolabeled solutions of pH 2, 3, 4 and 5 on addition of $^{68}GaCl_3$. In addition sodium acetate content was also optimized for kit formulation with specific volume of $^{68}GaCl_3$ (1 mL/2 mL/ 5 mL) (Table 1).

2.5.1. Kit A1: for 1 mL, 0.6 M HCl $^{68}GaCl_3$ (Generator A)

Varied volumes (350, 400, 450 and 500 μL) of 2 M sodium acetate were added to glass vials along with the peptide (40 μg/40 μL). Vials were then kept in dry ice for 30 min for freezing and subsequently placed for lyophilization in freeze-dryer (Scanvac Coolsafe, Denmark) at -50 °C for 6 h. Lyophilized kit vials were stored at 0 °C for further use for formulation with $^{68}GaCl_3$.

2.5.2. Kit A2: for 2 mL, 0.6 M HCl $^{68}GaCl_3$ (Generator A)

Volume of 2 M sodium acetate solution was doubled in kit preparation for this set. Sodium acetate solution (700, 800, 900 and 1000 μL) was added to vials along with the peptide, lyophilized and stored.