Nuclear Medicine and Biology 62–63 (2018) 47–53

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

# Gallium-68 labeled Ubiquicidin derived octapeptide as a potential infection imaging agent

Jyotsna Bhatt [a,c], Archana Mukherjee [a,c,*], Ajit Shinto [e], Kamaleshwaran Koramadai Karuppusamy [e], Aruna Korde [a,c,d], Mukesh Kumar [b,c], Haladhar Dev Sarma [b], Krishnamohan Repaka [d], Ashutosh Dash [a,c]

[a] Radiopharmaceuticals Division, Bhabha Atomic Research Centre (BARC), Mumbai 400085, India
[b] Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre (BARC), Mumbai 400085, India
[c] Homi Bhabha National Institute, Anushaktinagar, Mumbai 400094, India
[d] Quality control, Board of Radiation and Isotope Technology, Vashi, Navi Mumbai, India
[e] Dept of Nuclear Medicine and PET-CT, Kovai Medical Center and Hospital Limited, Coimbatore, India

ARTICLE INFO

*Article history:*
Received 24 January 2018
Received in revised form 23 March 2018
Accepted 27 April 2018
Available online xxxx

*Keywords:*
Antimicrobial peptides
Infection imaging
PET imaging
$^{68}$Ga radiopharmaceuticals
$^{68}$Ga-NOTA-UBI (29–41)
$^{68}$Ga-NOTA-UBI (31–38)

ABSTRACT

*Introduction:* Gallium-68 based infection imaging agents are in demand to detect infection foci with high spatial resolution and sensitivity. In this study, Ubiquicidin derived octapeptide, UBI (31–38) conjugated with macrocyclic chelator NOTA was radiolabeled with $^{68}$Ga to develop infection imaging agent.
*Methods:* Circular dichroism (CD) spectroscopy was performed to study conformational changes in UBI (31–38) and its NOTA conjugate in a "membrane like environment". Radiolabeling of NOTA-UBI (31–38) with $^{68}$Ga was optimized and quality control analysis was done by chromatography techniques. *In vitro* evaluation of $^{68}$Ga-NOTA-UBI (31–38) in *S. aureus* and preliminary biological evaluation in animal model of infection was studied. Initial clinical evaluation in three patients with suspected infection was carried out.
*Results:* $^{68}$Ga-NOTA-UBI (31–38) was prepared in high radiochemical yields and high radiochemical purity. *In vitro* evaluation of $^{68}$Ga-NOTA-UBI (31–38) complex in *S. aureus* confirmed specificity of the agent for bacteria. Biodistribution studies with $^{68}$Ga-NOTA-UBI (31–38) revealed specific uptake of the complex in infected muscle compared to inflamed muscle with T/NT ratio of 3.24 ± 0.7 at 1 h post-injection. Initial clinical evaluation in two patients with histopathologically confirmed infective foci conducted after intravenous injection of 130–185 MBq of $^{68}$Ga-NOTA-UBI (31–38) and imaging at 45–60 min post-injection revealed specific uptake at the sites of infection and clearance from vital organs. No uptake of tracer was observed in suspected infection foci in one patient, which was proven to be aseptic and served as negative control.
*Conclusion:* This is the first report on $^{68}$Ga labeled NOTA-UBI (31–38) fragment for prospective infection imaging.


## 1. Introduction

Peptides are gaining importance for clinical applications as drugs and as radiopharmaceuticals after labeling with suitable radionuclides [1,2]. A number of target specific peptide radiopharmaceuticals are now being used in clinics for detection and therapy of tumors and other diseased conditions. Due to their specificity towards the target, rapid clearance from blood and other non-target tissues contributed by small size and low antigenicity, peptides are very effective as targeting agents. The availability of different techniques to generate high-affinity peptides for a selected target is also responsible for the large pool of synthetic bioactive peptides. For development of molecular imaging agents, the most important advantage of peptides is their tolerance towards the modifications necessary for labeling with different radionuclides. For radiolabeling, the most explored approach makes use of a bifunctional chelating agent (BFCA) that coordinates the radionuclide and forms stable conjugate with the targeting peptide [3,4].

Molecular imaging to detect infection foci is still a challenge. An ideal infection imaging agent to detect different types of infection caused by different microorganisms with high specificity and sensitivity is yet not available [5]. Efforts are being made to develop infection imaging agent using small peptides specific towards infection causing microorganisms. Ubiquicidin, an antimicrobial peptide is reported to have specificity towards a number of Gram positive bacteria, Gram negative bacteria and fungi [6,7]. For infection imaging, the 29–41 fragment of Ubiquicidin (UBI), an antimicrobial peptide (AMP) labeled with $^{99m}$Tc has shown promising results in clinical management of conditions such as osteomyelitis, fever of unknown origin, suspected bone and prosthesis infection *etc.* [8–11].

* Corresponding author at: Radiopharamceuticals Evaluation Section, Radiopharmaceuticals Division, Bhabha Atomic Research Centre, Mumbai 400085, India.
*E-mail address:* archanas@barc.gov.in (A. Mukherjee).

https://doi.org/10.1016/j.nucmedbio.2018.04.003
0969-8051/

Gallium-68 based imaging agents are in demand due to inherent advantages of positron emission tomography (PET) imaging in comparison to single photon imaging (SPECT). $^{68}$Ga-citrate, $^{68}$Ga Apotransferrin and Desferri-triacetylfusarinine C (TAFC) and desferriferricrocin (FC) siderophores labeled with $^{68}$Ga are reported for imaging infection [12,13]. Several peptide analogues were designed for the visualization of vascular adhesion protein-1 (VAP-1) expressed on endothelial surface in inflammation and infection [14]. But none of these agents are specific for microorganisms causing infection.

The aim of the present study was to explore whether UBI (31–38), an octapeptide derived from Ubiquicidin which was proposed as a region important in binding to bacterial membranes could also serve the purpose of a specific infection imaging agent after radiolabeling similar to $^{68}$Ga labeled UBI (29–41) reported recently [15]. Conformational changes in UBI (31–38) peptide and its 1,4,7 triazacyclononane 1-glutaric acid 4–7 acetic acid (NOTA) conjugate were studied in a membrane like environment by circular dichroism (CD) spectroscopy [16]. Herein, we report labeling of UBI (31–38) conjugated with NOTA with $^{68}$Ga, characterization by chromatography methods, stability studies of the complex and *in vitro* evaluation in *S. aureus*. Preliminary biological evaluation of $^{68}$Ga-NOTA–UBI (31–38) in animal model of infection is also reported. Initial clinical evaluation in three patients with suspected infection was also carried out.

## 2. Materials & methods

UBI (31–38) with amino acid sequence Arg-Ala-Lys-Arg-Arg-Met-Gln-Tyr conjugated with macrocyclic chelator, NOTA was custom synthesized by CheMatech (Dijon, France). UBI (31–38) fragment was procured from ABI Scientific, USA. The 740 MBq $^{68}$Ge/$^{68}$Ga generator was purchased from iThemba Lab (Gauteng, South Africa). 0.6 N HCl used for elution of $^{68}$Ga from the generator was prepared using Suprapure HCl (30%) from Merck (Darmstadt, Germany). Sodium acetate (>99% purity, ACS, Reag, Ph Eur) was obtained from M/s. Merck KGaA (Darmstadt, Germany). High pressure liquid chromatography (HPLC) grade water from Merck (Mumbai, India) was used for preparation of all reagent solutions. Whatman 3 MM paper (Whatman, Maidstone England) and ITLC-SG paper from Agilent technologies (Lakefront, CA USA) were used for estimation of radiochemical yield (RCY) of the complexes. For *in vitro* studies, *S. aureus* (ATCC 25923) and Brain Heart Infusion Broth (BHI) were procured from Himedia (Mumbai, India). All the glass vials and rubber closures were acid washed and rinsed thoroughly in HPLC grade water prior to use.

Conformation of samples was studied using a Jasco J-815 CD spectrometer (Tokyo, Japan). Radioactivity measurements were made using a NaI (Tl) scintillation counter from ECIL (Hyderabad, India). An HPLC system from JASCO (Tokyo, Japan) equipped with a C-18 reversed phase HiQ Sil (5 μm, 4 × 250 mm) column was used for HPLC analyses. The system was coupled to a PU 1575 UV/visible detector, and a NaI (Tl) radioactivity detector from Raytest (Straubenhardt, Germany).

### 2.1. Circular dichroism spectroscopy (CD)

Conformations of peptide and conjugate were studied by CD spectroscopy. 100 μg of samples of UBI (31–38) and NOTA-UBI (31–38) were dissolved in 500 μL of 10 mM potassium phosphate buffer pH 7. Studies were also carried out in trifluoroethanol (TFE) in phosphate buffer as solvent to observe changes in conformation of peptide and peptide conjugate in different environments [16]. Optimization of percentage of TFE from 20 to 80% in buffer was carried out using UBI (31–38) peptide. Respective buffers were used as blank for the experiments. Background corrected CD signals were recorded for samples at wavelengths 250–180 nm. CD signal in millidegrees were plotted against the wavelengths for all the samples.

### 2.2. Optimization of radiolabeling protocol for NOTA-UBI (31–38)

Gallium-68 was eluted from 740 MBq (20 mCi) $^{68}$Ge/$^{68}$Ga generator. Fractional elution of $^{68}GaCl_3$ was carried out in 1 mL of 0.6 N HCl (five successive elutions of 1 mL each). $^{68}$Ga activity from second eluate was used for radiolabeling. Radiolabeling of NOTA-UBI (31–38) conjugate was optimized by varying concentration of peptide (14 nmol to 35 nmol), reaction time (5 min, 10 min), pH (3, 3.5, 4) and temperature of reaction (Room temperature (RT ~22 °C), 37 °C, 60 °C, 90 °C). Briefly, 200 μL, $^{68}GaCl_3$ (74 MBq) was added to peptide conjugates in 4 M sodium acetate to achieve pH 3.5. After optimization, incubation was carried out at 90 °C for 10 min.

### 2.3. Characterization of $^{68}$Ga labeled NOTA-UBI (31–38)

The radiochemical yield (RCY) of $^{68}$Ga-NOTA-UBI (31–38) was determined by ITLC and radiochemical purity was determined by high performance liquid chromatography (HPLC) technique. Aliquot of reaction mixture (~5 μL) were applied on ITLC-SG strips (10 × 1 cm). The strips were developed in 15% HCl in methanol and 0.1 M sodium citrate solution (pH 5). Radioactivity associated with strips was measured using NaI (Tl) counter.

HPLC analysis of the $^{68}$Ga-labeled UBI (31–38) complex was carried out using a dual pump HPLC unit with a C-18 reversed phase column. The elution was monitored by detecting UV signals at 214 nm and radioactivity signal using NaI (Tl) detector. Water (A) and acetonitrile (B) mixtures with 0.1% trifluoroacetic acid were used as the mobile phase and gradient elution (0–2 min: 5% B, 2–32 min: 65% B, 32–35 min: 5% B) was adopted for the separation of free $^{68}$Ga and $^{68}$Ga complexes. Flow rate was maintained at 1 mL/min.

### 2.4. Stability studies

Integrity of the $^{68}$Ga labeled peptide conjugates in saline and human serum was estimated by incubating 50 μL of complex in 450 μL of saline and serum in separate vials for 1 h at 37 °C. Aliquot was taken after 1 h and analysis was carried out by ITLC and HPLC technique. Stability of undiluted complex was also studied similarly for 2 h.

### 2.5. Partition coefficient estimation

Partition coefficient of the $^{68}$Ga-NOTA-UBI (31–38) complex was estimated by incubating 100 μL of complex with 500 μL of octanol and 400 μL of normal saline. The organic and aqueous layers were thoroughly mixed using vortex mixer. Organic and aqueous phases were separated by centrifugation at 3000 rpm for 5 min. Aliquots of samples from both the layers were taken and activity associated was counted using NaI (Tl) counter. Log *P* values were calculated by finding log of activity concentration in *n*-octanol/activity concentration in aqueous layer.

### 2.6. In vitro uptake assay

*S. aureus* (ATCC 25923) cells were grown overnight in BHI at 37 °C. After 24 h, cells were diluted in BHI to achieve optical density of 0.6 at 600 nm which correlates to $10^8$ colony forming units (CFU)/mL Optical density of culture was measured using UV–Vis spectrophotometer at 600 nm. ~$10^8$ CFU were used for bacterial uptake and inhibition studies. Bacterial cells were washed with 15 mM sodium phosphate buffer, pH 7.4. Uptake assay was carried out in an incubation buffer (15 mM sodium phosphate buffer, 0.01% tween (v/v) 80, 0.1% acetic acid (v/v), pH 5). Briefly, bacterial cells were incubated with $^{68}$Ga-NOTA-UBI (31–38) complex at final concentration of ~0.5 μM in the incubation buffer for 1 h at 37 °C with mixing. Inhibition studies were carried out by preincubation with ~ 100 fold excess of unlabeled UBI compared to tracer concentration for 1 h at 37 °C followed by addition of tracer.