Iran J Nucl Med. 2023;31(2):157-166 [Serial No. 61]

Homepage: https://irjnm.tums.ac.ir/

ORIGINAL RESEARCH ARTICLE

Development and evaluation of [^{64}Cu]Cu-DOTATATE for clinical applications

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ARTICLE INFO

Article History:
Received: 13 December 2022
Revised: 16 March 2023
Accepted: 18 March 2023
Published Online: 29 May 2023

Keyword:
Copper-64
Radiolabeled peptide
DOTATATE
PET-CT

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ABSTRACT

Introduction: Peptide-based radiopharmaceuticals have great advantages that make them one of the most interesting radiotracers for theranostic applications. This study aims to develop [^{64}Cu]Cu-DOTATATE as a beneficial agent for PET imaging of neuroendocrine tumors (NETs).

Methods: ^{64}Cu was produced via ^{68}Zn(p,αn)^{64}Cu reaction using 30 MeV Cyclotron. [^{64}Cu]Cu-DOTATATE was prepared at optimized labeling conditions by varying parameters. The radiochemical purity of [^{64}Cu]Cu-DOTATATE was checked by various methods. The stability of the final radiolabeled compound was assessed in PBS buffer and human serum. Binding affinity and internalization rate of [^{64}Cu]Cu-DOTATATE were studied on the Rat C6 glioma cell line. The biodistribution of [^{64}Cu]Cu-DOTATATE was studied in normal and tumor-bearing rats at different intervals. Finally, the images were taken after the administration of the radiopharmaceutical by a dual-head SPECT system.

Results: [^{64}Cu]Cu-DOTATATE was produced with radiochemical purity >99% (RTLC & HPLC) and specific activity of 22.4 GBq/mg in optimized conditions. [^{64}Cu]Cu-DOTATATE demonstrated high stability in vitro and in vivo. The binding studies showed a high binding affinity of the radiopharmaceutical to somatostatin-receptor-expressing cells. The internalization studies showed >58% of the radiopharmaceutical is internalized into the C6 cells within 6 h after incubation. The biodistribution of [^{64}Cu]Cu-DOTATATE in normal and tumor-bearing rats showed high uptake of somatostatin-receptor-expressing organs and tumors, respectively. The images of tumor-bearing rats were consistent with the results of the biodistribution study.

Conclusion: Preclinical studies of [^{64}Cu]Cu-DOTATATE showed that the radiopharmaceutical has a high potential for domestic use in PET imaging of patients with NETs.

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How to cite this article: Zolghadri S, Badipa F, Amraee N, Alirezapour B, Beiki D, Yousefnia H. Development and evaluation of [^{64}Cu]Cu-DOTATATE for clinical applications. Iran J Nucl Med. 2023;31(2):157-166.

https://doi.org/10.22034/IRJNM.2023.129117.1543

INTRODUCTION

Neuroendocrine tumors (NETs) originate from endocrine tissues throughout the body that contain large numbers of high-affinity somatostatin receptors (SSRs). Radiopharmaceuticals are used with very high reliability in the diagnosis, therapy, and treatment follow-up by nuclear medicine procedures [1]. Single photon emission computed tomography (SPECT) and positron emission tomography (PET) can provide the visualization and quantification of functions at the cellular or molecular level [2].

For more than three decades, peptide receptor-based scintigraphy has been accepted as a valuable diagnostic method in nuclear medicine procedures [3]. [123I]I-Tyr3-octreotide was first used in patients to study human NET in 1989 [4]. Due to some limitations of labeled compounds based on 123I, [111In]In-DTPA-D-Phe1-octreotide ([111In]In-pentetreotide) was replaced and used in many patients in 1993 [5] and finally was approved in 1994 by the U.S. Food and Drug Administration (FDA) as a NET imaging agent. However, there were still limitations, such as low spatial resolution, moderate affinity for receptors, and high gamma energy regarding this radiopharmaceutical. For all these reasons, the researchers focused on other radioelements such as technetium-99m for single photon emission computed tomography (SPECT) and gallium-68 for positron emission tomography (PET). However, clinical studies on three labeled compounds, [^{68}Ga]Ga-DOTATOC, [^{68}Ga]Ga-DOTATATE, and [^{68}Ga]Ga-DOTANOC have shown that they are very good radiotracers and are now routinely used in clinical applications, the relative advantages of [^{68}Ga]Ga-DOTATATE in PET imaging of NET have been proven [6, 7].

Copper-64 (^{64}Cu), with a half-life of 12.7 h that emits β+ [19%] and β− [38%] particles, is known as one of the suitable PET radioisotopes for diagnosing and treating various types of cancers. The ^{64}Cu half-life demonstrates high flexibility to bind with different kinds of small and large molecules to provide various diagnostic and therapeutic radiolabeled compounds based on ^{64}Cu radioisotope [8, 9] such as [^{64}Cu]Cu-ATSM [10, 11], [^{64}Cu]Cu-Trastuzumab [12, 13], [^{64}Cu]Cu-Cetuximab [14], [^{64}Cu]Cu-AE105 [15, 16], [^{64}Cu]Cu-PSMA-617 [17], etc.

Among the various clinical studies that have been conducted on ^{64}Cu-somatostatin analogs, [^{64}Cu]Cu-DOTATATE was one of the first ones used. The results of clinical studies have shown that the diagnostic value of [^{64}Cu]Cu-DOTATATE in patients with NET is significantly better than [111In]In-DTPA-octreotide [18]. Also, the advantages of [^{64}Cu]Cu-DOTATATE in detecting lesions in NET patients compared to [^{68}Ga]Ga-DOTATOC have been well-proven [19].

Due to the increasing incidence of malignant diseases specifically with neuroendocrine origin throughout the world, and the outstanding characteristics of [^{64}Cu]Cu-DOTATATE in the detection of these tumors, this study is aimed to prepare [^{64}Cu]Cu-DOTATATE radiopharmaceutical in optimized conditions and to complete a thorough preclinical study to domestically produce a reliable [^{64}Cu]-based radiopharmaceutical for clinical application in patients with neuroendocrine tumors.

METHODS

^{64}Cu was produced via ^{68}Zn(p,αn)^{64}Cu reaction using 30 MeV Cyclotron (Cyclone-30, IBA, Belgium). DOTATATE peptide was obtained from ABX Co. (Radeberg, Germany). FBS (Fetal Bovine Albumin) and RPMI cell culture medium were purchased from GIBCO Co. (England). All other chemical reagents were provided by Sigma Aldrich (Heidelberg, Germany). Radio-chromatography was performed using Whatman No. 1 paper (Whatman, U.K.) and a thin-layer chromatography scanner (Bioscan AR2000, Paris, France). The activity of the samples was measured by an N-type coaxial high-purity germanium (HPGe) detector (NIGC-4020) coupled with a multichannel analyzer card system (NIGC1040-, DSG, GMBH). The C6 glioma mouse cell line was purchased from Pasteur Institute (Iran). The Student's T-test was used to compare the data based on statistical significance defined as $P < 0.05$.

Copper-64 production

For copper-64 production, the zinc-68 (^{68}Zn) target was electroplated on a high-purity gold-coated copper backing plate. The target with a thickness of 100 µm was irradiated by an approximately 30 MeV proton beam at an angle of 6 degrees to achieve a higher production yield. The target was cooled by a flow of 18°C distilled water, with a rate of 50 Lit/min. The target was dissolved in 15 mL, 10 N HCl containing 20 µL of hydrogen peroxide and passed through a cation exchange column. The cation exchange column was washed with 25 mL, 9 N HCl at a speed of 1 mL/min to remove copper and zinc. After dilution, this mixture was passed through an anion exchange column, and finally,

copper-64 was washed from the column with 50 mL of 2 N HCl.

Quality control of [^{64}Cu]CuCl$_2$

The radionuclide purity of the final sample was measured by gamma spectroscopy using an HPGe detector. The area under the curve for the observed peaks was counted for at least 1000 seconds. The radiochemical purity of the product was checked by the RTLC method in the presence of two solvent systems of 1 mM diethylenetriaminepentaacetic acid (DTPA; C14H23N3O10) and, 10% ammonium acetate: methanol (1:1) as the mobile phases on Whatman paper No. 2 and silica gel as the stationary phases, respectively. The chemical purity of the product was evaluated by determining the concentration of copper and zinc cations using polarographic techniques.

Preparation and quality control of [^{64}Cu]Cu-DOTATATE

The stock solution of DOTATATE peptide in distilled water (1 mg/ml) was prepared for labeling purposes. To determine the optimized labeling conditions, various parameters such as reaction pH, peptide concentration, reaction time, etc., were changed, and in each case, the radiochemical purity of the radiolabeled compound was determined.

The radiochemical purity of [^{64}Cu]Cu-DOTATATE radiolabeled compound was checked by HPLC and RTLC methods. In the RTLC method, silica gel and Whatman papers were considered as the stationary phases, and ammonium hydroxide 10%: methanol: water (1:5:10; V:V:V), and ammonium acetate 10%: methanol (30:70; V:V) mixtures were used as the mobile phases. For HPLC analysis, a C18ODS column with dimensions of 100 mm × 4.6 mm filled with 5 µm particles was used. The column was washed with mobile phases of A: Ultrapure water + 1% TFA and, B: Acetonitrile, with a flow rate of 2.6 mL.min-1 using gradient-elution: 0-3 min, A:100%, B: 0%; 3- 10 min, A:50%, B: 50%; 10-15 min, A:0%, B:100%.

Stability studies

The stability of the final radiolabeled compound was checked in PBS buffer and human blood serum. A sample of [^{64}Cu]Cu-DOTATATE (37 MBq) was kept in PBS buffer (4 ˚C) for about one half-life of Cu-64, and the radiochemical purity of the sample was evaluated using the RTLC method at 2, 4, 6, and 12 h. In addition, to check the stability in human serum, 500 µL of fresh serum was added to a sample of the compound and it was kept at 37 degrees for about one half-life of copper-64. During this period, at 2, 4, 6, and 12 hours, the radiochemical purity of the sample was evaluated using the RTLC method.

Binding affinity test

The binding affinity of [^{64}Cu]Cu-DOTATATE was studied according to the published protocols with some slight modifications [20, 21]. Briefly, 0.8-1×106 cells expressing somatostatin, i.e., Rat C6 glioma cell line, were seeded inside each well of a 6-well plate and incubated overnight with complete culture medium in an incubator. After 24 h, the culture medium was removed, and the cells were washed and incubated with the freshly prepared culture medium for 1 h at 37˚C. The plates were placed on ice for 30 min, and different concentrations (1-100 nM) of ^{64}Cu-DOTATATE were added to PBS buffer (pH=7.4). Non-specific binding of the radiolabeled peptide was estimated in the presence of 1 µM of cold peptide for 30 min at 37 °C. The cells were rewashed with ice-cold PBS, and then the cells were harvested, and the bound activity was measured using a gamma counter. The total concentration of SSTR receptors expressed on C6 glioma cells (Bmax), and the dissociation constant (Kd) were estimated by non-linear regression obtained using Graph-Pad software (Prism 8 Graph Pad Software, San Diego, CA).

Internalization studies

The internalization rate of the radiolabeled peptide was also estimated on C6 glioma cells according to the published procedures with some slight modifications [20,21]. Briefly, 1×105 cells were seeded in each well of the 6-well plates and incubated overnight for about 24 h in a CO2 incubator. [^{64}Cu]Cu-DOTATATE (2.5 pmol) was added to each well and was incubated for 30, 60, 120, 240, and 360 min at 37 ˚C. The culture medium was removed and the cells were washed and incubated twice for 5 min with 1 mL glycine buffer for dissociation of surface-bound radiolabeled peptide and then cells were rinsed twice with ice PBS. Finally, 1 mL NaOH solution (1 N) was added to the wells and the culture medium was removed to measure internalized activity. The radiolabeled peptide bound to the surface and the internalized part was measured using a gamma counter.

Biodistribution studies of [^{64}Cu]CuCl$_2$ and [^{64}Cu]Cu-DOTATATE in normal rats

[^{64}Cu]CuCl$_2$ and [^{64}Cu]Cu-DOTATATE (150 µL; 5.55 MBq) were injected into the normal Sprague-Dawely rats weighing 140-160 gr; aged 8-10 weeks via their tail veins. Rats were sacrificed at 2, 4, 12, and 24 h after injection and

the main organs including the liver, spleen, kidney, stomach, small intestine, large intestine, heart, muscle, lung, pancreas, and bone were removed, rinsed, and the injected dose per gram (ID/g%) for each organ was calculated using Equation (1):

$$\frac{ID}{g}\% = \frac{A(t)}{A_0 \times m} \qquad (1)$$

Where A(t) is the organ activity in Time (t) measured according to Equation (2) [22], A_0 is the injected activity and m is the organ mass (gr).

$$A = \frac{N}{\varepsilon\, \gamma\, ts\, k1\, k2\, k3\, k4\, k5} \qquad (2)$$

Where ε is the efficiency at photopeak energy, γ is the emission probability of the gamma line corresponding to the peak energy, ts is the lifetime of the sample spectrum collection in seconds, m is the mass (kg) of the measured sample, k1, k2, k3, k4, and k5 are the correction factors for the nuclide decay from the time the sample is collected to start the measurement, the nuclide decay during counting period, self-attenuation in the measured sample, pulses loss due to random summing and the coincidence, respectively. Where N is the corrected net peak area of the corresponding photo-peak given as:

$$N = Ns\frac{ts}{tb}\, Nb \qquad (3)$$

Where Ns is the net peak area in the sample spectrum, Nb is the corresponding net peak area in the background spectrum, and ts is the lifetime of the background spectrum collection in seconds.

Biodistribution studies of [^{64}Cu]Cu-DOTATATE in tumor-bearing rats

Tumors were implemented by subcutaneously injecting the C6 cells (1×107/100 µL in PBS) to the right flank of 6-8 weeks male rats. The biodistribution study in tumor-bearing rats was started when the average tumor volume reached 800–1000 mm3. [^{64}Cu]Cu-DOTATATE (5.55 MBq/150 µL) was injected into tumor-bearing rats through the tail veins and the biodistribution was studied at 2, 4, 12, and 24 hours post-injection. Blocking studies were performed by injection of 100 µg/200 µL cold peptide into the tumor-bearing rats at 4 h post-injection. Also, the biodistribution of [^{64}Cu]$CuCl_2$ in tumor-bearing rats was assessed 4 h post-injection. The %ID/g for each organ was calculated after the activity measurement using Equation (1).

Imaging studies

[^{64}Cu]Cu-DOTATATE (5.55 MBq/150 µL) was injected into tumor-bearing rats through the tail veins and images were taken 4, and 24 h after administration of the radiopharmaceutical by a dual-head SPECT system. The rat-to-high-energy-septa distance and the useful field of view (UFOV) were considered 12 cm and 540 mm × 400 mm, respectively.

RESULTS

Preparation and quality control of [^{64}Cu]$CuCl_2$

Copper-64 was produced via ^{68}Zn(p,αn)^{64}Cu reaction in a 30 MeV cyclotron and was converted to [^{64}Cu]$CuCl_2$ by dissolving in HCl. Radionuclide purity of the final sample measured by an HPGe detector showed that all three observed energy peaks are related to copper-64 (278 keV; 9%, 511 keV; 35%, and 1346 keV; 1%). The absence of other peaks indicated a very high radionuclide purity of the final product (> 99.9%). The radiochemical purity assessment of [^{64}Cu]$CuCl_2$ in two solvent systems of 1 mM DTPA, and 10% ammonium acetate: methanol (1:1) demonstrated a purity of >99% (Figure 1). Evaluation of the chemical purity using polarographic techniques showed that copper (0.81±0.12 ppm) and zinc (0.73±0.16 ppm) in the final product were less than 1 ppm, which in addition to being within the permissible limit, does not cause a serious problem for the labeling of peptides.

Preparation and quality control of [^{64}Cu]Cu-DOTATATE

The optimized labeling conditions were determined as follows: 500 µL sodium acetate

buffer (pH=5.5) and 15 µg DOTATATE was added to the vial containing 370 MBq [^{64}Cu]$CuCl_2$. The reaction was continued at 95 °C for 20 min. The radiochemical purity of [^{64}Cu]Cu-DOTATATE checked by HPLC and RTLC methods demonstrated a purity of >99% (Figures 2 & 3).

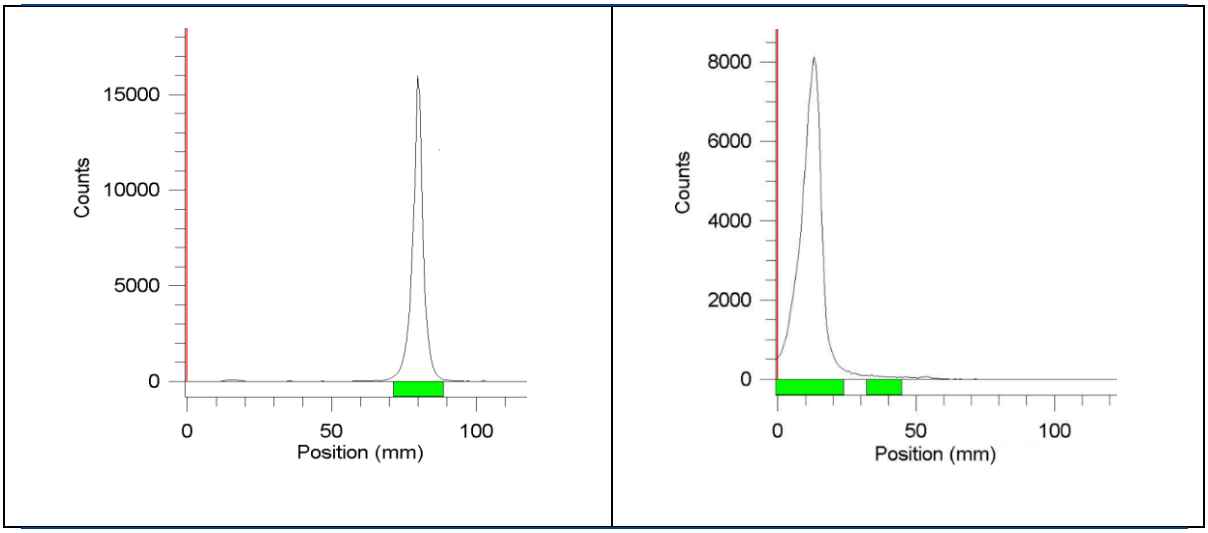

Fig 1. Radiochromatogram of [^{64}Cu]CuCl2 in 1 mM DTPA (left) and in 10% ammonium acetate: methanol ratio (1:1))

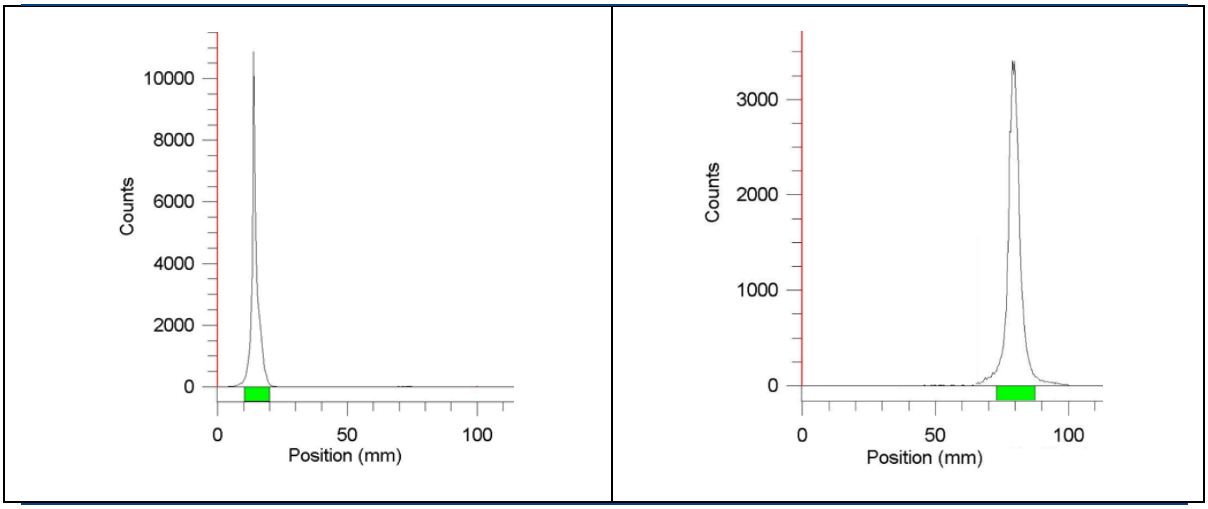

Fig 2. Radiochromatogram of [^{64}Cu]Cu-DOTATATE in ammonium acetate 10%: methanol (30:70; V:V))

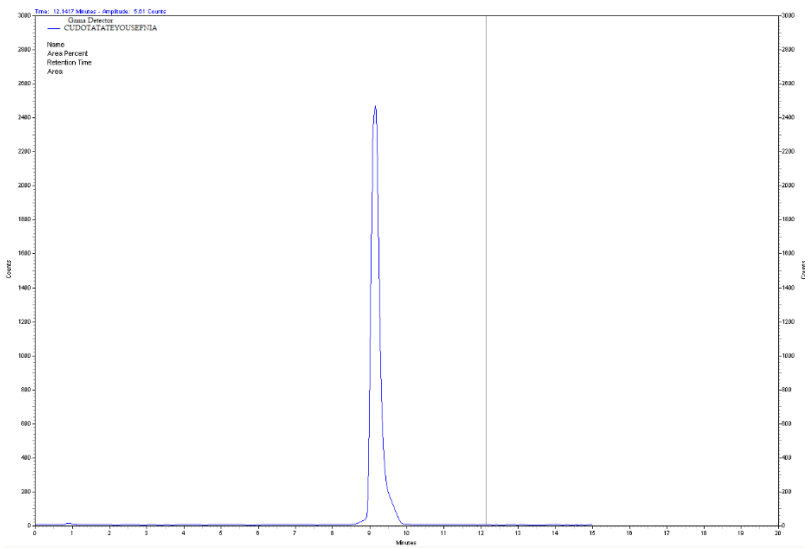

Fig 3. HPLC chromatogram of [^{64}Cu]Cu-DOTATATE

Stability studies

The stability tests of the final radiolabeled compound were checked by the radiochemical purity measurements showing the radiochemical purity of 95.5 %± 1.8% and 94.9% ± 1.7% after 12 h, in PBS buffer (4 ˚C) and human blood serum, respectively.

Binding affinity study

The total concentration of SSTR receptors expressed on C6 glioma cells (Bmax) and the dissociation constant (Kd) was performed with rat C6 glioma cells. The saturation binding curve was drowned for [^{64}Cu]Cu-DOTATATE and the Kd value was determined (Figure 4).

Internalization studies

The internalization rate of [^{64}Cu]Cu-DOTATATE was estimated on C6 glioma cells at different intervals post-treatment. The results showed the internalization increased from about 16% in 30 min to >58% in 6 h post-treatment.

Biodistribution studies of [^{64}Cu]$CuCl_2$ and [^{64}Cu]Cu-DOTATATE in normal rats

Biodistribution of [^{64}Cu]$CuCl_2$ and the radiolabeled compound in the normal rats was investigated at 2, 4, 12, and 24 h after injection. The % ID/g for each organ after injection of [^{64}Cu]$CuCl_2$ and [^{64}Cu]Cu-DOTATATE is indicated in Figures 5 & 6, respectively.

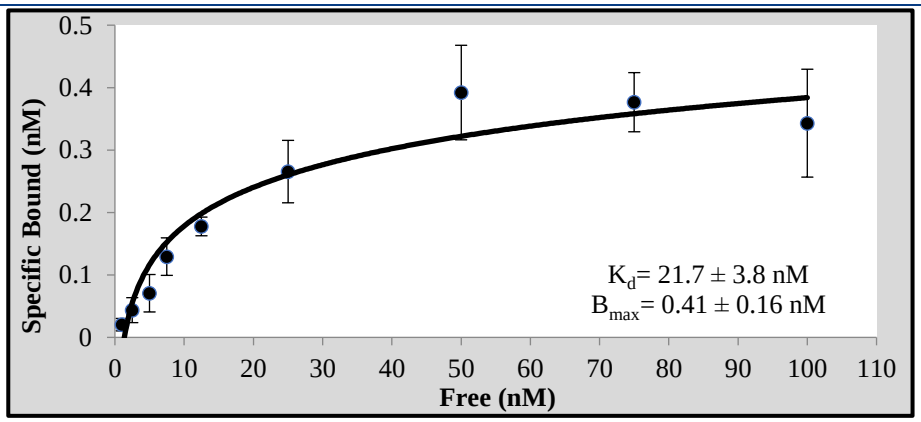

Fig 4. Saturation binding curve for [^{64}Cu]Cu-DOTATATE

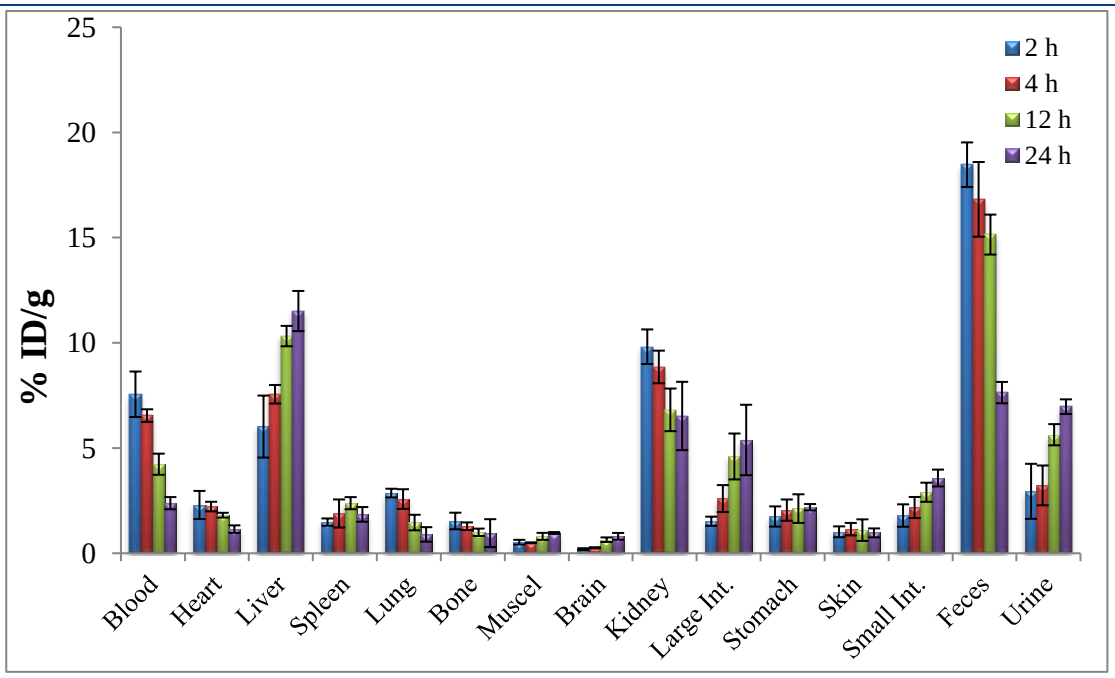

Fig 5. Biodistribution of [^{64}Cu]$CuCl_2$ in normal rats (n = 3)

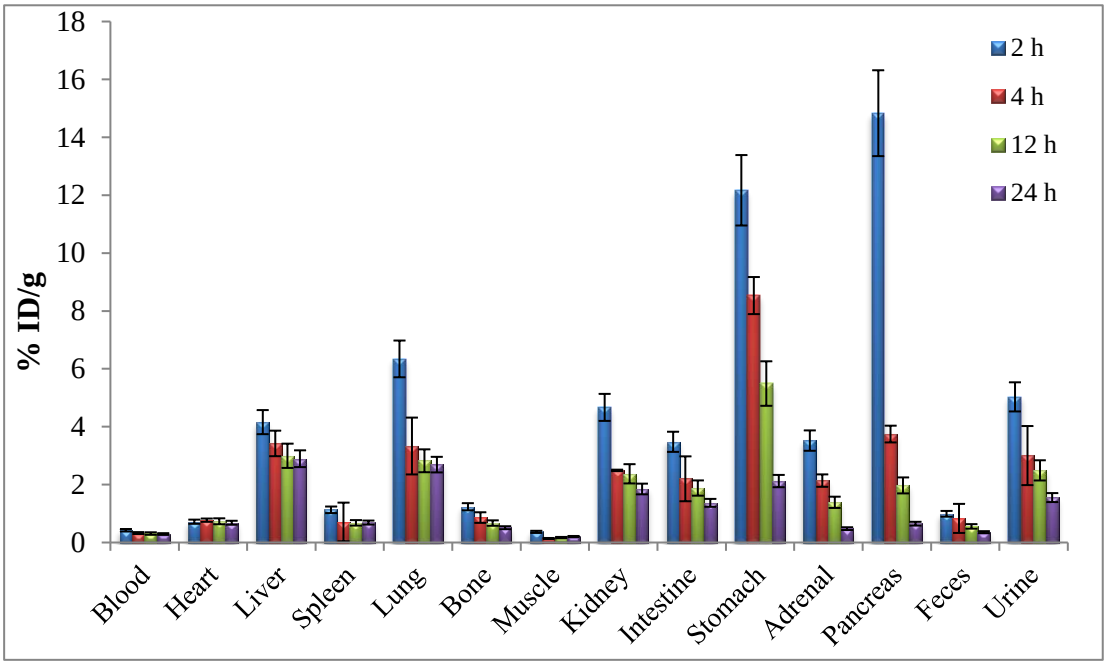

Fig 6. Biodistribution of [^{64}Cu]Cu-DOTATATE in normal rats (n = 3)

Biodistribution studies of [^{64}Cu]Cu-DOTATATE in tumor-bearing rats

The biodistribution of [^{64}Cu]Cu-DOTATATE in tumor-bearing rats was studied at 2, 4, 12, and 24 h post-injection. The biodistribution of [^{64}Cu]$CuCl_2$ in tumor-bearing rats and the blocking study in tumor-bearing rats were performed at 4 h post-injection (Table 1).

Table 1. Biodistribution of [^{64}Cu]Cu-DOTATATE in tumor-bearing rats (mean ± SD) (P value < 0.05)

Organ	2h	4h	4h blocking	4h Free ^{64}Cu	12h	24h
Blood	0.53 ± 0.12	0.37 ± 0.11	0.39 ± 0.19	6.01 ± 1.27	0.31 ± 0.13	0.26 ± 0.09
Heart	0.96 ± 0.15	0.85 ± 0.23	0.98 ± 0.27	2.87 ± 0.79	0.79 ± 0.17	0.81 ± 0.21
Liver	4.75 ± 1.03	3.86 ± 0.67	3.75 ± 0.53	7.01 ± 0.84	2.63 ± 0.42	2.05 ± 0.36
Spleen	1.32 ± 0.44	0.91 ± 0.30	0.82 ± 0.36	1.63 ± 0.45	0.86 ± 0.34	0.55 ± 0.27
Lung	5.43 ± 1.62	4.01 ± 1.14	3.68 ± 0.93	2.44 ± 0.39	3.11 ± 0.95	2.84 ± 0.34
Bone	1.16 ± 0.33	0.92 ± 0.26	0.70 ± 0.21	1.14 ± 0.24	0.61 ± 0.18	0.49 ± 0.14
Muscle	0.47 ± 0.10	0.18 ± 0.04	0.21 ± 0.06	0.53 ± 0.16	0.13 ± 0.03	0.07 ± 0.01
Kidney	4.1 ± 0.60	2.94 ± 0.46	2.66 ± 0.51	7.43 ± 1.52	2.54 ± 0.39	1.98 ± 0.41
Intestine	3.81 ± 0.34	2.65 ± 0.38	2.30 ± 0.42	2.77 ± 0.35	1.71 ± 0.27	1.59 ± 0.32
Stomach	13.23 ± 2.12	8.15 ± 0.88	3.27 ± 0.48	1.96 ± 0.28	4.24 ± 1.12	2.81 ± 0.52
Adrenal	3.67 ± 0.93	2.39 ± 0.72	1.14 ± 0.33	1.14 ± 0.33	1.63 ± 0.69	0.58 ± 0.22
Pancreas	13.81 ± 2.18	3.24 ± 0.82	1.01 ± 0.26	1.01 ± 0.26	1.57 ± 0.36	0.83 ± 0.17
Feces	1.20 ± 0.22	0.96 ± 0.24	0.82 ± 0.31	12.63 ± 3.13	0.49 ± 0.19	0.28 ± 0.09
Urine	4.89 ± 0.71	3.41 ± 0.48	2.93 ± 0.65	3.45 ± 0.72	2.21 ± 0.33	1.45 ± 0.46
Tumor	11.76 ± 1.39	10.04 ± 1.09	1.29 ± 0.31	0.89 ± 0.21	3.15 ± 0.73	2.30 ± 0.51

Imaging studies

[^{64}Cu]$CuCl_2$ and [^{64}Cu]Cu-DOTATATE (5.55 MBq/150 µL) were injected into tumor-bearing rats through the tail veins and images were 4, and 24 h after administration of the radiopharmaceutical by a dual-head SPECT system (Figures 7 & 8). The rat-to-high-energy-septa distance and the useful field of view (UFOV) were considered 12 cm and 540 mm × 400 mm, respectively.

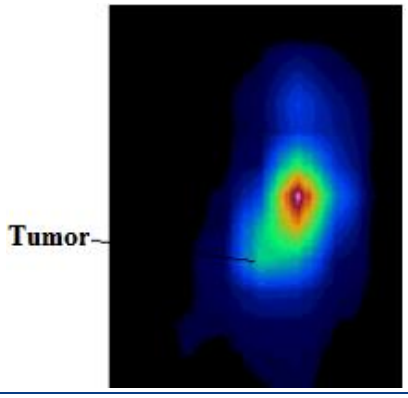

Fig 7. Scintigraphic image of the tumor-bearing rat, 4 h post-injection of [^{64}Cu]$CuCl_2$

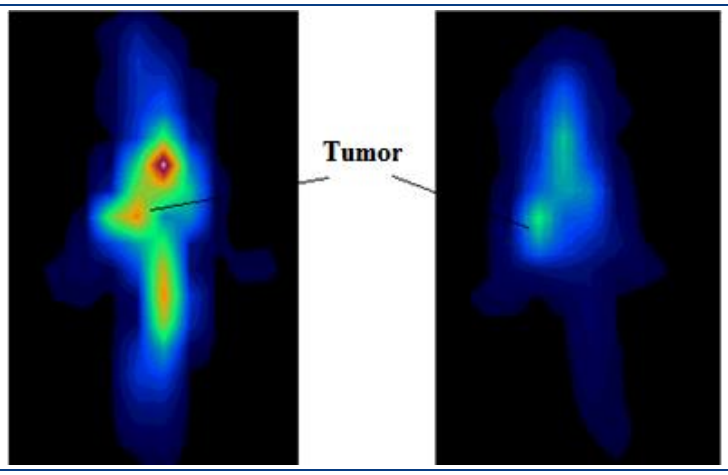

Fig 8. Scintigraphic images of the tumor-bearing rats, 4 h (left) and 24 h (right) post injection of [^{64}Cu]Cu-DOTATATE

DISCUSSION

Neuroendocrine tumors (NETs) are often small and highly variable and can occur in any part of the body not uncommonly with metastatic involvement at presentation, making their diagnosis a challenging issue [23]. Today, new radiopharmaceuticals based on peptides and antibodies have been developed for the targeted diagnosis as well as targeted therapy of tumors. In principle, somatostatin analogs conjugated chelators such as DOTATATE labeled with the PET (e.g., ^{68}Ga or ^{64}Cu), SPECT (e.g., ^{111}In), or (e.g., ^{177}Lu) therapeutic radioisotopes are used for diagnostic and therapeutic purposes, respectively [24]. The use of ^{111}In-DTPA-octreotide is still common, especially in North America. However, due to the significant advantages of PET imaging over SPECT imaging, [^{68}Ga]Ga-DOTATATE was introduced as a new PET radiotracer and was approved by U.S. FDA for NETs PET imaging in June 2016 and [^{64}Cu]Cu-DOTATATE was recently approved by FDA as a new SSTR PET imaging agent. ^{64}Cu has lower positron ranges compared to ^{68}Ga, which theoretically results in better spatial resolution. Also, the longer physical half-life of ^{64}Cu (12.7 h) compared to ^{68}Ga (68 min) provides the possibility of imaging smaller tumors within optimal time limits [19]. In a head-to-head comparison clinical evaluation, [^{64}Cu]Cu-DOTATATE has detected significantly more true-positive lesions in patients with NET compared to [^{68}Ga]Ga-DOTATOC (82.5% vs. 17.5%) and also a higher detection rate of NET has been reported for [^{64}Cu]Cu-DOTATATE compared to [^{68}Ga]Ga-DOTATOC [19]. Different independent studies demonstrated that various SST analogs labeled with ^{64}Cu could be safely used for the diagnosis of NETs [25]. Also, today [^{64}Cu]Cu-DOTATATE has been known as a reproducible, highly accurate, and practical agent for NET detection and metastases with superiority over other radiolabeled SST analogs [25].

Due to the special characteristics of [^{64}Cu]Cu-DOTATATE in the detection of NET, this radiopharmaceutical with radiochemical purity >99% (RTLC & HPLC) and specific activity of 22.4 GBq/mg in optimized conditions were prepared for domestic use. As expected, [^{64}Cu]Cu-

DOTATATE demonstrated very high stability in vitro and in vivo.

One of the most important factors in the investigation of a radioligand is to evaluate its binding affinity to the receptors. The maximal binding capacity of the radiopharmaceutical was obtained Bmax = 0.41 ± 0.16 nM, while the dissociation constant of [^{64}Cu]Cu-DOTATATE was calculated to be Kd = 21.7 ± 3.8 nM. Bmax and Kd for 64/natCu-DOTA-TATE determined on HEK-hsst2 cells were reported 0.48 ± 0.18 nM and 20.1 ± 4.4 nM in another study [26], which is almost comparable with our study. Cell binding study for [^{64}Cu]Cu-DOTATATE on C6 cells showed high binding affinity of the radiopharmaceutical to cells expressing somatostatin receptors in vitro. Internalization studies of [^{64}Cu]Cu-DOTATATE on C6 cells demonstrated that more than 58% of the radiopharmaceutical is internalized to the cells within 6 h after treatment that is in accordance with the agonistic nature of the DOTATATE and therefore with the function of the radiopharmaceutical [26].

The biodistribution of [^{64}Cu]$CuCl_2$ in normal rats showed that free copper-64 had the highest accumulation in the liver, kidney, and intestine in all intervals post-injection and the highest amount of free copper-64 excretion was through feces. As copper is an essential component in different enzymatic processes, it is absorbed in the liver and upper intestine and from the liver, either excreted into the bile or released into the systematic circulation [27, 28]. The highest accumulation of copper-64 in the liver is in agreement with other copper-64 distribution data [29].

The amount of [^{64}Cu]Cu-DOTATATE in the blood was significantly lower than the amount of free copper-64 in all intervals (P value < 0.005), which indicates the rapid clearance of the radiopharmaceutical from the blood. In addition, high accumulation of the radiopharmaceutical in the organs with somatostatin receptors, such as the pancreas, indicates organ-specific accumulation of the radiopharmaceutical, which is in accordance with other DOTATATE radiolabeled agents [30]. The accumulation of [^{64}Cu]Cu-DOTATATE in the kidney as well as the high accumulation of activity in the urine show that the urinary tract is the main route of excretion for the radiopharmaceutical, in accordance with the excretion of other radiopeptides [30, 31].

The biodistribution of the radiopharmaceutical in tumor-bearing rats showed the high accumulation of the radiopharmaceutical in the organs overexpressing somatostatin receptors as well as the tumor site, which confirmed the high specificity of the radiopharmaceutical towards the tumor. The generated images after the injection of [^{64}Cu]Cu-DOTATATE to the tumor-bearing rats with somatostatin receptors showed that the radiopharmaceutical had a high accumulation at the tumor site, which is entirely in agreement with the results of clinical trials data of this radiopharmaceutical [32].

This study has potential limitations. The specific activity of the final product strongly depends on the chemical purity of the produced copper-64 and its chemical purity depends on the production and radiochemical separation processes. On the other hand, the limitations of the number of tumor-bearing mice may affect the errors and the statistical analyses.

CONCLUSION

[^{64}Cu]Cu-DOTATATE was produced with radiochemical purity >99% (RTLC & HPLC) and specific activity of 22.4 GBq/mg in optimized conditions. The preclinical studies of the radiopharmaceutical, including cellular assays and biodistribution and imaging studies in normal and tumor-bearing rats, showed that the radiopharmaceutical has a high potential for domestic use in PET imaging of patients with NETs.

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