Available online at www.sciencedirect.com
ScienceDirect
Nuclear Science and Techniques 19 (2008) 159–164
NUCLEAR SCIENCE AND TECHNIQUES

Biodistribution study of [^{61}Cu]pyruvaldehyde-bis (N-4-methylthiosemicarbazone) in normal rats as a PET tracer

Amir Reza JALILIAN[1*] Saeed SHANESAZZADEH[2] Pejaman ROWSHANFARZAD[1]
Fatemeh BOLOURINOVIN[1] Abbas MAJDABADI[1]

[1]*Nuclear Medicine Research Group, Agriculture, Medicine and Industrial Research School (AMIRS), Karaj, Iran;*

[2]*Department of Medical Physics, Tehran University of Medical Sciences, Tehran, Iran*

Abstract [^{61}Cu]-labeled pyruvaldehyde-bis(N-4-methylthiosemicarbazone) (^{61}Cu-PTSM), a promising agent made for imaging blood perfusion, was produced via the ^{nat}Zn(p,x)^{61}Cu nuclear reaction in a 30 MeV cyclotron, and separated by a two-step column chromatography method developed in our laboratory using a cation and an anion exchange resin. After 150 μA irradiation for 76 min, about 6.006 Ci of $^{61}Cu^{2+}$ was obtained with a radiochemical separation yield of 95% and a radionuclidic purity of 99%. ^{61}Cu-PTSM was prepared using an optimized method with in-house synthesized PTSM ligand for radiolabeling following quality control procedures using RTLC and HPLC. The tracer is mostly incorporated in heart, kidneys and brain compared to free copper cation as a control. These are in agreement with former reports. In conclusion, [^{61}Cu]-PTSM was prepared at the radiopharmaceutical scales with high quality and is a potential PET tracer in the perfusion study of the heart, kidney, brain and tumors.

Key words Copper-61, PTSM, Biodistribution, Radiolabeling

CLC numbers R817.4, O621.3^{+}5

1 Introduction

Positron emission tomography (PET) is a powerful diagnostic tool in nuclear medicine. Having been engaged in radiosynthesis and evaluation of PET radiopharmaceuticals [1-3], we were interested in the production of other PET tracers such as ^{61}Cu to develope some novel tumor seeking radiopharmaceuticals.

Copper offers a unique selection of radioisotopes (^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, and ^{67}Cu) with half-lives ranging from 9.8 min to 61.9 h suitable for imaging and/or radiotherapy. The most commonly used copper radioisotopes, ^{62}Cu and ^{64}Cu, provide very good physical properties for therapeutic and/or diagnostic purposes. Few production methods of ^{61}Cu have been reported for radiolabeling of biomolecules and other applications [4-6]. Interestingly, it has been shown that the tomographic images obtained using ^{61}Cu are superior to those using ^{64}Cu, as ^{61}Cu emits more positrons than ^{64}Cu [7]. ^{61}Cu has been used in radiolabelling of small hypoxic imaging molecules [8] for various diagnostic purposes. Recently we reported the production [9] and use of various ^{61}Cu labeled tracers for PET imaging of hypoxia [10], blood cells[11] and malignancies [12].

Cu-pyruvaldehyde-bis(N-4-methylthiosemicarbazone ([Cu]-PTSM) was used in internal radiation therapy and imaging in late 1980's [13,14] and has been used since then in the determination of regional blood flow and renal perfusion [15-17], tumor blood flow [14], ischemia [16,18]and finally radiotherapy of tumors [19,20].

Based on the interesting imaging properties of

*Corresponding author. E-mail: ajalilian@nrcam.org

Received date: 2008-01-24

^{61}Cu-PTSM and its production via ^{nat}Zn(p,x)^{61}Cu reaction as a by-product at our 30MeV cyclotron, we were interested in biological distribution of [^{61}Cu]-PTSM as a possible PET tracer (Fig. 1) in blood flow imaging.

2 Materials and methods

Production of ^{61}Cu was performed in the AMIRS 30 MeV cyclotron (IBA, Cyclone-30). Natural zinc oxide of 98% purity was commercially available. Chemicals were purchased from Aldrich Chemical Company (Milwaukee, U.S.A.). Thin layer chromatography (TLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20 × 20 cm, TLC Ready Foil, Schleicher & Schuell® Germany). Methanol and normal saline used for labeling were of high purity. Radio-chromatography was performed by polymer-backed silica gel paper and/or C_{18} thin layer sheets using a thin layer chromatography scanner (Bioscan AR2000, Paris, France). Radionuclide purity was checked by the same detector. All calculations and TLC counting were based on 511 keV peak. A Beckman DU® 640 Spectrophotometer (U.S.A.) was used for colorimetric assays. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edition.

2.1 Targetry and bombardment

The target was a layer of natural zinc, electroplated on copper plate which was coated with a 50-μm gold layer to prevent interference of the backing copper during radiochemical separation. Cross section calculations by ALICE nuclear code [21] showed that the best proton energy range for ^{nat}Zn(p,x)^{61}Cu reaction is 22-12 MeV. The target had to be thick enough to reduce the proton energy from 22 MeV to about 12 MeV. The targets were irradiated in a galncing angle of 6º to achieve higher production yield. SRIM code [22] was run to determine the best target thickness in the energy range.

2.2 Gold and zinc electrodeposition

A gold containing bath was prepared according to Ref. [23] with slight modifications. As the 6° glancing angle reduces the required target thickness by 10 fold, electroplating a 75-μm thick target is good enough. The target was irradiated by 22 MeV (150 μA) protons for 76 min.

2.3 Chemical separation

Chemical separation was carried out in no-carrier-added form. The irradiated target was dissolved in 10 mol/L HCl (15 mL, H_2O_2 added). The solution passed through a cation exchange resin (AG 50W, H^+ form, mesh 200-400, 1.3×10 cm) which had been preconditioned by passing 25 mL of 9 mol/L HCl. The column was then washed by 25 mL of 9 mol/L HCl with a rate of 1 mL/min to remove copper and zinc ion contents. To the eluent 30 mL water was added to about 100 mL of a 6 mol/L HCl solution [24]. The latter solution was loaded on another exchange resin (AG1X8 Cl^- form, 100-200 mesh, 25×1.7 cm) pretreated with 6 mol/L HCl (100 mL). Finally, ^{61}Cu was eluted using 2 mol/L HCl (50 mL) in form of [^{61}Cu]$CuCl_2$. The whole process took about 60 min[25].

2.4 Preparation of pyruvaldehyde-bis (N-4-methylthiosemicarbazone)

PTSM was prepared according to Ref. [26] for thiosemicarbazone production. A mixture of N-4-methylthiosemicarbazide (210 mg, 2 mmol) in acetic acid solution (5%, prepared with 99% AcOH and MilliQ-H_2O) was heated to 50°C with stirring until a transparent solution was formed. Freshly distilled pyruvaldehyde (115 mg, 2 mmol) diluted (1:3) with 5% acetic acid was added drop-wise to the mixture in 5 min under a blanket of N_2. The mixture was stirred for 3~4 h at 50°C. The hot reaction mixture was filtered off through two layers of Whatman No.2 filter paper. The filtered mass was washed with MilliQ-H_2O (50 mL), rectified ethanol (25 mL) and was finally heated in a vacuum oven overnight at 75°C. The dried powder was refluxed in 80% acetic acid (prepared with MilliQ-H_2O) for 2 h. The hot mixture was filtered and the precipitate was washed with MilliQ-H_2O (50 mL), rectified ethanol (25 mL) and was heated at a vacuum oven overnight at 75°C.

2.5 Preparation of [^{61}Cu]pyruvaldehyde-bis(N-4-methylthiosemicarbazone)

[^{61}Cu]$CuCl_2$ (3 mCi) dissolved in acidic medium obtained above (about 2 mL) was transferred to a 5 mL-vial containing 3 mol/L (4 mL) sodium acetate. A mixture of pyruvaldehyde-bis (N-4-methylthiosemicarbazone) (3 μg) in absolute ethanol (0.1 mL) [8] was added to the copper acetate solution and vortexed at 50°C for 3~5 min. The mixture (about 5 mL) was then cooled in an ice bath, and injected rapidly into a C_{18} Sep-Pak column pretreated with 5 mL of ethanol and 2 mL of water. The column was washed with water (4 mL) and purged with a stream of dry N_2. The labeled compound was finally eluted using 0.2 mL-portions of absolute ethanol and the fractions were counted with an HPGe detector. The vial containing the maximum radioactivity was diluted to a 5% solution by addition of normal saline. The active solution was checked for radiochemical purity by polymer-backed silica gel layer chromatography using dry ethyl acetate as mobile phase. The final solution passed through a 0.22 μm filter and pH was adjusted to 5.5~7 by adding 3 mol/L sodium acetate buffer.

2.6 Chemical purity

The formation of colored dithizone-zinc complex was measured using visible spectroscopic assay to determine Zn cation concentrations according to Ref. [27] using dithizone organic reagent (0.002% in CCl_4). Briefly, the presence of pinkish color of zinc-dithizone complex was checked for the test sample, 1, 5, 10μg/g standards and finally a blank solution (1 mL each). The color of the test tube must be paler than that of the standard.

2.7 Radiochemical purity

Radio thin layer chromatography was performed using a mixture of dry ethyl acetate as the mobile phase for both pre-column and post-column fractions using Bioscan detector. The step motor was installed to count 0.4-cm-piece each 30 second through a slot of a shielded chamber. Radiochemical yields were determined by comparing the un-complexed ^{61}Cu (R_f=0.0) and the major radio peak at R_f= 0.80.

2.8 Stability of [^{61}Cu]PTSM complex in the final product

Stability studies were based on the previous studies performed for radiolabeled copper complexes. A sample of [^{61}Cu]PTSM (0.5 mCi) was kept at room temperature for 5 h while checked by RTLC every 30 min. A micropipet sample (5 μL) was taken from the shaking mixture and the ratio of free radiocopper to [^{61}Cu]PTSM was checked by radio thin layer chromatography (eluent: dry ethyl acetate).

2.9 Stability of [^{61}Cu]PTSM complex in presence of serum

A mixture of 5 parts of serum and one part radiopharmaceutical (0.2 mCi) was shaken in a 37º incubator under N_2 atmosphere. A micropipet sample (5 μL) was taken from the shaking mixture every 30 minutes. The ratio of free radiocopper (R_f=0) to [^{61}Cu]PTSM (R_f=0.8) was checked by radio thin layer chromatography (eluent: dry ethyl acetate).

2.10 Biodistribution of [^{61}Cu]PTSM in normal rats

To determine its biodistribution, [^{61}Cu]PTSM was administered to normal rats (NMRI) purchased from Razi Institute, Karaj, Iran. A volume (50 μL) of final [^{61}Cu]PTSM solution containing 40±1 μCi radioactivity was injected intravenously to rats through their tail vein. The animals were sacrificed at exact time intervals (1,2 and 3 h), and the specific activity of different organs was calculated as percentage of ID/g (calculated from the area under 511 keV peak using an HPGe detector). At each time interval 5 animals were sacrificed and the average of 5 uptakes was with at most 2% of SD.

3 Results and discussion

3.1 Targetry and irradiation

For 76 min bombardment of the ^{nat}Zn target with 22 MeV proton, 150 μA, the resulting activity of ^{61}Cu was 222 GBq (6.0 Ci) at the end of bombardment (E.O.B.) and the production yield was 440 MBq/μAh. Yield from the radiochemical separation was more than 95%. Quality control of the product was performed in two steps. Radionuclidic control showed

the presence of 67.41(4.23%), 282.96(12.2%), 373(2.15%), 511(122.9%), 656(10.77%), 1186(3.75%) keV γ-rays from ^{61}Cu and showed a radionuclidic purity higher than 99% (E.O.S.). The rest of activity was attributed to ^{60}Cu (0.23%). In order to check the chemical purity, concentration of zinc (from target material) and gold (from target support) were determined using visible colorimetric assays. The presence of zinc cations was checked by visible colorimetric assays. Even at 1 μg/g of standard zinc concentration, the pinkish complex was visible by naked eye, while the test sample remained similar to the blank. The colorimetric assay demonstrated that the zinc cation concentration was far below the maximum permitted levels, i.e. 5 μg/g (less than 1 μg/g zinc). The gold concentration was less than 0.9 μg/g.

3.2 Preparation and structure confirmation of the ligand

Pyruvaldehyde-bis (N-4-methylthiosemicarbazone), not commercially available, was prepared according to the general procedure of thiosemicarbazone preparation[26]. The reaction was performed in 5% acetic acid solution containing N-4-methylthiosemi- carbazide. Fig. 1 shows the route to prepare the ligand and labeled compound.

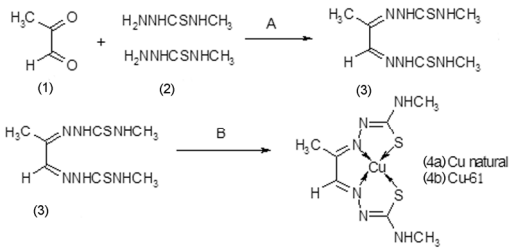

Fig. 1 Schematic diagram of the preparation method for PTSM (3) and [^{61}Cu]PTSM (4b). A.5% AcOH, 50°C; B. [^{61}Cu]CuOAc, C_{18} Sep-Pak.

3.3 Radionuclidic purity

The final product was in radionuclidic purity of over 96% by measuring the 511 and 283 keV γ-rays from ^{61}Cu.

3.4 Radiolabeling of pyruvaldehyde-bis(N-4-methylthiosemicarbazone)

The freshly eluted ^{61}Cu chloride solution was changed into copper acetate using a 3 mol/L sodium acetate solution, with pH 5.5~7 for complex formation. The ligand 3, dissolved in absolute ethanol, was added to the buffered solution so that a final 2% concentration of ethanol was obtained. This procedure was superior to the former labeling procedure using DMSO as the ligand solvent [11]. The mixture was vortexed in a tube shaker and left at room temperature for 5~10 min. The solid phase separation of the ^{61}Cu-PTSM from free copper cation was performed to obtain a better purity and the lipophilic complex was eluted using 0.2-mL absolute ethanol fractions (Fig. 2).

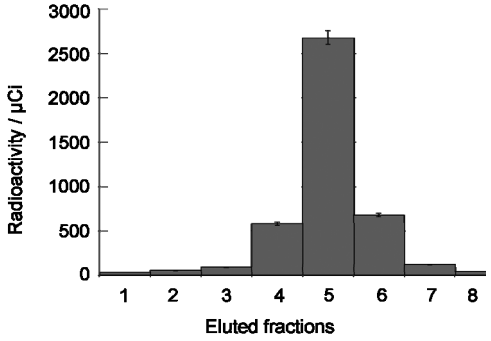

Fig. 2 The activity of 0.2-mL ethanol fractions used in tracer SPE on C_{18} Sep-Pak.

3.5 Radiochemical purity

Labeling PTSM with copper cation affects its chromatographic properties and the final complex is highly lipophilic. Thus free copper and the labeled PTSM can easily be separated using solid phase C_{18} Sep-Pak column. In the TLC studies, the more polar un-complexed PTSM and free copper fractions correlate to smaller R_f (R_f=0.0).

The complexed PTSM migrates at the higher R_f (R_f=0.8). In all radiolabeling runs (n=9), after solid phase extraction of the labeled mixture, the integral ratio of the two peaks were constant (98:2), showing the high radiochemical purity (Fig. 3).

3.6 Formulation and stability

The final radiolabeled complex diluted in normal saline then passed through a 0.22 μm filter (Millipore) when filtration was used to sterilize the product. Due to its thermal instability, [^{61}Cu]PTSM preparation could totally be degraded and left detectable amounts of free copper after autoclaving.

The chemical stability of [^{61}Cu]PTSM was high enough to perform further studies. The final product RTLC was stable, showing no changes in the patterns for trace [^{61}Cu]CuOAc and [^{61}Cu]PTSM in 5 h.

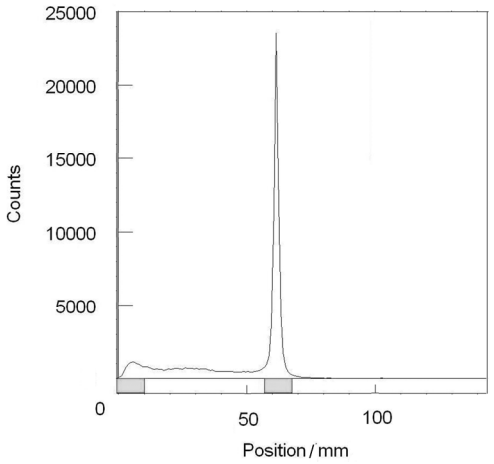

Fig. 3 RTLC diagram of 98% pure [^{61}Cu]PTSM.

3.7 Biodistribution studies

The biodistribution studies were performed 1h (Fig. 4), 2h (Fig. 5) and 3h (Fig. 6) post injection of the tracer in normal rats.

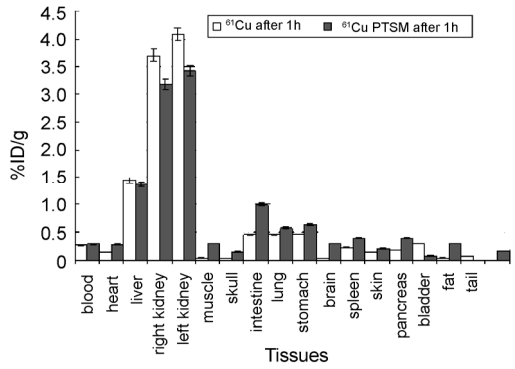

Fig.4 Bio-distibution data for [^{61}Cu]$CuCl_2$ and [^{61}Cu]PTSM in normal rats 1 h post-injection of 40μCi of the tracer (*n*=3).

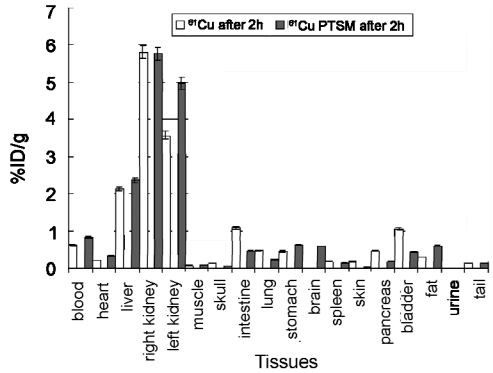

Fig.5 Bio-distibution data for [^{61}Cu]$CuCl_2$ and [^{61}Cu]PTSM in normal rats 2 h post-injection of 40μCi of the tracer (*n*=3).

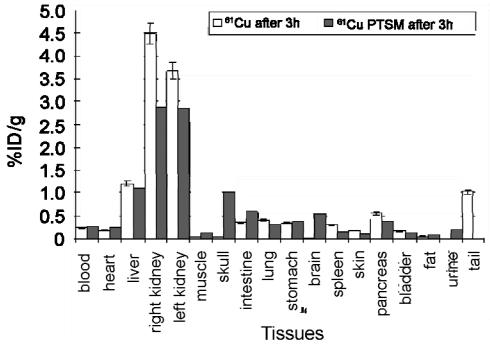

Fig.6 Bio-distibution data for [^{61}Cu]$CuCl_2$ and [^{61}Cu]PTSM in normal rats 3 h post-injection of 40μCi of the tracer (*n*=3).

In human studies [28], it was shown that the tracer accumulates in liver, spleen and heart and in less extend in brain. This was in agreement with our observations in this study.

4 Conclusion

Total labeling and formulation of [^{61}Cu]PTSM took about 10 min, with a yield of 97%~98%. A suitable specific activity product was formed *via* insertion of [^{61}Cu]copper cation. No labeled by-products were observed upon RTLC analysis of the final preparations after solid phase extraction (SPE) purification. The radio-labeled complex was stable in aqueous solutions for at least 5 h and no significant amount of other radioactive species were detected by RTLC. Trace amounts of [^{61}Cu]copper acetate (≈2%) were detected by RTLC. The radiochemical purity of the [^{61}Cu]PTSM was higher than 98%. [^{61}Cu]PTSM is a PET radiotracer with an intermediate half life, and the high chemical stability of this radiopharmaceutical makes it a very suitable diagnostic agent to be sent to clinic.

References

1 Jalilian AR, Seyfi P, Afarideh H, *et al*. Int. J Appl Radiat Isot. 2000, **5**: 545-555.

2 Jalilian AR, Afarideh H, Shafiee A, *et al*. J Pharm Pharmaceut. *Sci.* 2000; **23**: 114-124.

3 Jalilian AR, Afarideh H, Shafiee A, *et al*. J Labelled Compd Radiopharm. 2001, **44**: 11-21.

4 Tolmachev V, Lundqvist H, Einarsson L. Int J Appl Radiat Isot. 1998, **49**: 79-81.

5 McCarthy DW, Bass LA, Cutler PD, *et al.* Nucl Med Biol. 1999, **26**: 351-358.

6 Ma DF, Lu T, *et al*. Nucl Med Biol. 2002; **29**: 91-105

7 Fukumura T, Okada K, Szelecsényi F, *et al*. Radiochimica Acta. 2004, **92**: 209-214.

8 Lewis JS, Sharp TL, Laforest R, *et al*. J Nucl Med. 2001, **42**: 655-661.

9 Rowshanfarzad P, Sabet M, Jalilian AR, *et al*. Applied Radiation and Isotopes, 2006, **64**:1563–1573.

10 Jalilian AR, Sabet M, Rowshanfarzad P, *et al*. Journal of Radioanalytical and Nuclear Chemistry, 2006, **269**: 147–154.

11 Jalilian AR, Rowshanfarzad P, Sabet M. Nukleonika, 2006, **51**: 111-117.

12 Jalilian AR, Rowshanfarzad P, Sabet M, *et al*. Applied Radiation and Isotopes, 2006, **64**: 337-341.

13 Green MA. Nucl Med Biol 1987, **14**:59-61.

14 Marczenko Z. Spectrophotometric determination of elements, 4th Ed. Warsaw:Ellis Horwood Ltd., 1976:601-603.

15 Kostyniak PJ, Nakeeb SM, Schopp EM, *et al*. J Appl Toxicol, 1990, **10**:417-421.

16 Shelton ME, Green MA, Mathias CJ, *et al*. Circulation, 1990, **82**:990-997.

17 Young H, Carnochan P, Zweit J, *et al*. Eur J Nucl Med, 1994, **21**:336-341.

18 Young H, Carnochan P, Zweit J, *et al*. J Nucl Biol Med, 1994, **38**:89-91.

19 Wada K, Fujibayashi Y, Taniuchi H, *et al*. Nucl Med Biol, 1994, **21**:613-617.

20 Jason SL, Laforest R, Buettner TL, *et al*. Proc Natl Acad Sci USA, 2001, **98**:1206–1211.

21 Blann M, Bislinghoff J, Code Alice/Livermore 91, Lawrence Livermore National Laboratory, Internal Report, UCID-19614 (1991).

22 Ziegler JF, Biersack JP, Littmark U. The stopping and range of ions in matter (SRIM Code). Version 2000 XX, 2000.

23 Weisberg A M, Gold plating, 9th Ed., ASM International, U.S.A, 1990:247.

24 Hilgers K, Stoll T, Skakun Y, *et al*. Appl Radiat Isot, 2003, **59**:343-351.

25 Boothe TE, Tavano E, Munoz J, *et al*. J Label Comp Radiopharm, 1991, **30**:108.

26 Gingras BA, Suprunchuk T, Bayley CH. Can J Chem, 1962, **40**:1053-1057.

27 Marczenko Z. Spectrophotometric determination of elements, 4th Ed., Chichester: Ellis Horwood Ltd., 1976:369.

28 Wallhaus TR, Lacy J, Whang J, *et al*. J Nucl Med, 1998, **39**:1958–1964.