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Preparation and preliminary evaluation of [^{55}Co](II)vancomycin

Amir Reza JALILIAN * Yousef YARI-KAMRANI Pejman ROWSHANFARZAD
Mahseed SABET Mohsen KAMALI-DEHGHAN Abbas MAJDABADI

Nuclear Medicine Research Group, Agriculture, Medicine and Industrial Research School (AMIRS), Karaj, Iran

Abstract Co-55 ($t_{1/2}$=17.53 h) was produced by 150 μA irradiation of a natural nickel target using 15 MeV protons. It was separated from the irradiated target material by two ion exchange chromatography steps with a radiochemical yield of >95% and was used for the preparation of [^{55}Co]vancomycin ([^{55}Co]VAN). Optimization studies were performed using Co-57 due to its longer half-life. Cobalt-57 ($t_{1/2}$=271.79 d) was produced by irradiation of a natural nickel target with 150 μA current of 22 MeV protons. The ^{57}Co was separated from the irradiated target material using a no-carrier-added method with a radiochemical yield of >97%. Both products were controlled for radionuclide and chemical purity. The solutions of [^{55}Co]VAN were prepared (radiochemical yield>80%) starting with ^{55}Co acetate and vancomycin at room temperature after 30 min. A precise solid phrase extraction (SPE) method was developed using Si Sep-Pak in order to purify/reconstitute the final formulation for animal studies. [^{55}Co]VAN showed a radiochemical purity of more than 99%. The resultant specific activity was about 1.15 TBq/mmol. It is proved that the tracer is stable in the final product and in presence of human serum at 37°C up to 24 h. Biodistribution study of [^{55}Co]VAN in normal rats was undertaken for up to 72 h.

Key words Cobalt-55, Vancomycin, Medical cyclotron, Radiolabeling, Biodistribution

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1 Introduction

Cobalt-57, of 100% E.C. decay with $t_{1/2}$ = 271.9 d, has been used in preparation of various compounds with medical applications such as bleomycin used in detecting head and neck cancers [1], hydroxyl cobalamine used in pernicious anemia [2], Co-EDTA used in renographic studies [3] and Co-transferrin used in susceptible tumors [4]. The other cobalt radioisotope, *i.e.* ^{55}Co, a PET radionuclide of 60% β^+ decay and 40% E.C. decay with $t_{1/2}$ = 17.53 h, has been directly used in ^{55}Co-$CoCl_2$ form in detecting carotid artery disease [5], vascular dementia [6], renographic studies [7] and stroke [8] using PET scanners. To date, fewer ^{55}Co-labeled compounds are reported in the literature.

The emerging need for detecting infection sites in immunocompromised patients, has led to the introduction of various radiopharmaceuticals during the last two decades, including ^{99m}Tc-ciprofloxacin [9], radiolabeled human polyclonal antibodies [10] and radiolabeled chemotactic peptides [11]. Among these compounds, radiolabeled antibiotics demonstrate a significant discrimination between infection and inflammation sites.

Vancomycin hydrochloride is an antibiotic produced by certain strains of *Streptomyces orientalis*. Vancomycin HCl has the chemical formula $C_{66}H_{75}Cl_{12}N_9O_{24}$ HCl and a formula weight of 1486. During the first 24 hours, about 75% of an administered dose of vancomycin is excreted in urine by glomerular filtration. Fig. 1 shows the chemical structure of vancomycin.

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* Corresponding author. *E-mail address:* arjalilian@yahoo.com

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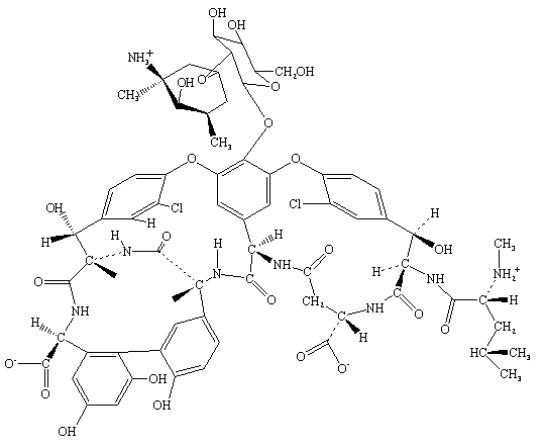

Fig.1 Chemical structure of vancomycin.

There is no apparent metabolism of the drug. The bactericidal action of vancomycin results primarily from inhibition of cell-wall biosynthesis. In addition, vancomycin alters bacterial-cell membrane permeability and RNA synthesis. There is no cross resistance between vancomycin and other antibiotics. Vancomycin is active against staphylococci, including *Staphylococcus aureus*. These properties suggest a suitable molecular imaging agent based on the vancomycin core [12].

Vancomycin metal complexes including Cu, Fe and Co have demonstrated similar biological activities compared to the native molecule, and the activity has enhanced in some cases such as the cobalt complex. The results have proved that vancomycin readily forms a complex with cobalt, and that once formed, the complex can be easily isolated chemically from the starting materials using size exclusion hromatography (SEC)[13]. The structure of cobalt-vancomycin complex has not been fully determined but it has been demonstrated cobalt interacts with the proximal phenolic hydroxyl groups on residues 5 and 6 [13].

Some groups used radiolabelled vancomycins for drug interaction investigations and ^{99m}Tc-vancomycin for possible infection imaging [14]. Based on our previous experiences on the production and biological evaluation of ^{201}Tl(III)vancomycin [15] and vast clinical application of vancomycin for therapeutic purposes [16], we were interested in the preparation of ^{55}Co-vancomycin as a possible PET infection imaging agent.

The imaging properties of ^{55}Co, which can be produced *via* ^{58}Ni(p,α)^{55}Co reaction, inspired the idea of providing this radionuclide in our laboratory for its ultimate use in the radiolabeling of vancomycin. We optimized [^{55}Co]VAN formation conditions, followed by stability studies of the complex in presence of human serum up to 15 h. Finally, the optimized tracer was administered to normal rats for biodistribution studies.

2 Materials and methods

Production of ^{55}Co and ^{57}Co was performed at a 30 MeV cyclotron (Cyclone-30, IBA). All chemicals were of analytical grade and were purchased from Merck Chemical Co. (Darmstadt, Germany). The ion-exchange resins were from Bio-Rad Laboratories, Canada. Radio-thin-layer-chromatography(RTLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20×20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). Normal saline and sodium acetate used for labeling were of high purity and were filtered through 0.22 μm Cativex filters. Radio- chromatography was performed by counting 5-mm portions of the strip using a scanner equipped with a Canberra™ HPGe detector (GC1020-7500SL). Radionuclide purities were checked using the same detector. All calculations and RTLC counts were based on the 477.2 keV peak.

2.1 Targetry

SRIM code was run to optimize target thickness [17] for ^{57}Co or ^{55}Co production. Projectile range of protons in nickel is illustrated in Fig. 2. A target thickness of 643.4 μm is required to reduce the beam energy from 22 MeV to 10 MeV, which is suitable for ^{57}Co production, and a 294.75 μm target reduces the beam energy from 15 MeV to 8 MeV for ^{55}Co production. The targets are fixed on special shuttles and sent to the target room by a rabbit system. The shuttles are designed to place the targets at an angle of 6° against the beam. Therefore, a 64 μm and a 30 μm layer of natural nickel were enough for producing ^{57}Co and ^{55}Co with desirable efficiency, respectively. The efficiency is higher, because the targets can endure higher currents with better heat transfer.

The natural nickel target was electrodeposited on a gold-plated copper backing, in order to avoid the contamination with troublesome copper removal chemistry. The methods used for electrodeposition of nickel and gold were based on two previous works [18,19]. The electrodeposition was carried out using a special cell which gave a surface area of about 1 cm ×11.58 cm.

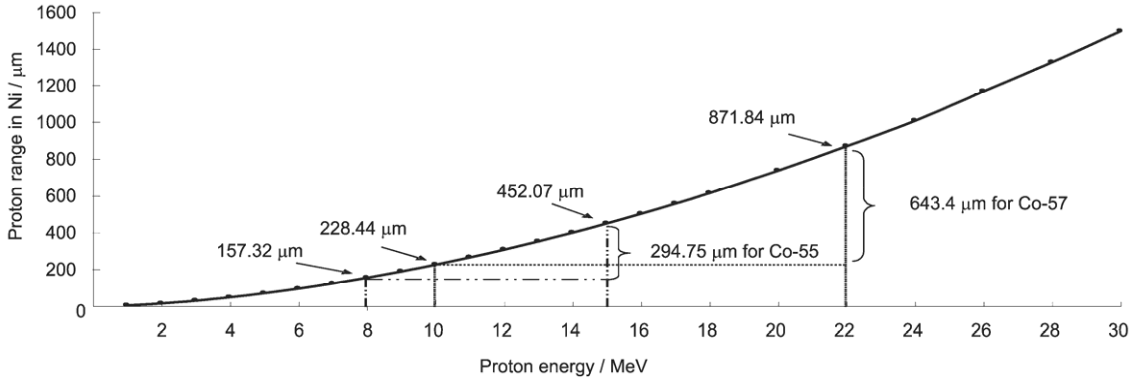

Fig. 2 Determination of the best target thickness for the production of ^{55}Co and ^{57}Co.

2.2 Production of ^{57}Co and ^{55}Co

The target was bombarded by a 150 μA 22 MeV protons. Radiochemical separation was carried out in no-carrier-added form [20,21] after two weeks cooling of the target for the decay of ^{57}Ni to ^{57}Co and eliminating short lived radionuclide impurities. The irradiated target was dissolved in refluxing warm 7 mol/L HNO_3 (30 mL) for 30 min. The solution was heated to dryness and the latter process was repeated after adding 20 mL distilled water. The residue was reconstituted by 9 mol/L HCl (20 mL) and the solution passed through an anion exchange resin (AG 1X8, Cl^- form, 100~200 mesh, Φ 1 cm × 8 cm) preconditioned with 25 mL of 9 mol/L HCl. The column was washed by passing 50 mL of 9 mol/L HCl at a rate of 1 mL/min to remove nickel ions. Finally, the ^{57}Co was eluted as ^{57}Co-$CoCl_2$ using 4 mol/L HCl (50 mL). The whole process took about 3 h.

The procedure used for the targetry and bombardment for ^{55}Co production was similar to that of ^{57}Co, except the target thickness and beam energy. The radiochemical separation process including ^{57}Co recovery from Ni and Cu was carried out immediately after the target bombardment. The recovery of Co and Cu from Ni was carried out in the same way as above for radiochemical separation of ^{57}Co. The only differences were using 20 mL of refluxing 7 mol/L warm HNO_3 in the first step and 20 mL of 4 mol/L HCl for the recovery of radiocobalt and radiocopper ions.

The eluent was evaporated to dryness and dissolved in 25 mL of 0.3 mol/L HCl-94% ethanol and was injected to an anion exchange resin (AG 1×8, Cl^- form, 100–200 mesh, Φ1 cm × 5 cm) pre-equilibrated with 25 mL of 0.3 mol/L HCl-82% ethanol. More than 95% of cobalt ions were recovered as ^{55}Co-$CoCl_2$ by passing 25 mL of 0.3 mol/L HCl-72% ethanol through the resin [22]. The whole process took about 4 h.

2.3 Product purity

Radionuclide purities of the products were controlled by a Canberra™ spectroscopy. The spectrum peaks were recorded for 3 h with ^{57}Co samples, and 1 h with ^{55}Co. Since the targets were natural nickel on gold backing, the presence of nickel cation was investigated using visible colorimetric assays. The most important photometric reagents for determining nickel are dioximes, which give specific and fairly sensitive methods. Dimethylglyoxime reacts with nickel ions in a neutral or ammoniacal medium to form a pink, flocculent precipitate. Even at 2 μg/g of standard nickel concentration, the colored Ni-dimethylglyoxime complex is visible to naked eye [23]. The amount of gold cation was controlled in the final solution using color formation with acidic rhodamine B reagent based on a previously reported colorimetric method [24].

2.4 Labeling of vancomycin with [^{55,57}Co]$CoCl_2$

[^{55}Co]$CoCl_2$ (185 MBq) dissolved in acidic medium (0.5 mL) was transferred to a 2 mL-borosilicate vial. The mixture was evaporated by slight warming (50°C) under a N_2 flow. An isotonic mixture of VAN (0.25 mg, 0.16 μmol/L) in Milli-Q® water (50 μL) was added to the residual activity and the vial was shaken for 30 s. The vial was left, heated and agitated at 25, 50, 80 or 100°C for 1 h to find out the best conditions. It was finally cooled to room temperature. The active solution was checked for radiochemical yield by polymer-backed silica gel layer chromatography. The mixture was injected into a Si Sep-Pak® column followed by elution using 0.9% NaCl solution. Eluted fractions (1mL each) were checked by RTLC/ITLC for radiochemical purity. The final selected fraction(s) was (were) combined through a 0.22 μm filter, and pH was adjusted to 5.5~7.0 if necessary.

Radio thin layer chromatography was performed using anhydrous ethanol as the eluent. Alternatively, normal saline could be used as the eluent, too. The radiolabeling yield and radiochemical purity were determined by comparing the un-complexed ^{55}Co (II) to the labeled compound radio peak using RTLC method.

A sample of [^{55}Co] VAN (18.5 MBq) was kept at room temperature for 5 days, while checked by RTLC every two hours. A micro-pipette sample (5 μL) was taken from the shaking mixture and the ratio of free radio-cobalt (II) to [^{55}Co] VAN was checked by radio thin layer chromatography(eluent: anhydrous ethanol).

Freshly prepared human serum of 500 μL was added to 36.1MBq (976μCi) of [^{57}Co] VAN. The mixture was incubated at 37°C, and 1.5-μL aliquots were analyzed by radio-TLC after 0, 1, 16.5, 18, 21 and 132 h of incubation to determine the complex stability.

2.5 Biodistribution in normal rats

Animal studies were performed in accordance with the United Kingdom Biological Council's "Guidelines on the Use of Living Animals in Scientific Investigations (2nd ed.). The distribution of [^{55}Co] VAN among tissues was determined for healthy NMRI male rats immediately after imaging. The total amount of activity injected into each rat was measured by counting the 1-mL syringe before and after injection in a dose calibrator with fixed geometry. The animals were sacrificed by CO_2 asphyxiation at 1, 3 or 52 h after injection. The tissues (blood, heart, lung, brain, intestine, urine, skin, stomach, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline. The activity per gram tissue was determined by detecting the 477.2 keV peak.

3 Results and discussion

3.1 Production and quality control of ^{57}Co and ^{55}Co

The production yield of ^{57}Co was 1.22 $MBq \cdot \mu A^{-1} \cdot h^{-1}$ and the radionuclide purity was 97.5% two weeks after the bombardment. The rest of activity was attributed to ^{56}Co. In the chemical control process, no nickel ions were detected under a detection limit of 2 μg/g. The test sample remained similar to the blank after the addition of 1% dimethylglyoxime to the alkaline sample previously treated with ammonia [25]. Gold concentration was confirmed to be less than 0.9 μg/g. According to many toxicological databases, nickel cation LD_{50} value for rat is 67 μg/g [26]. Thus this method might not be the best analytical method for Ni^{2+} detection but it is definitely far below LD_{50} of Ni cation (33 times better).

The production yield of ^{55}Co was 10 $MBq \cdot \mu A^{-1} \cdot h^{-1}$ at the end of bombardment. And the radionuclide purity was over 99.3%. The rest of activity was attributed to ^{57}Co. In the chemical control process, no nickel ions were detected under a detection limit of 2 μg/g. Gold concentration was confirmed to be less than 0.9 μg/g. Radiochromatography of ^{55}Co in various solvents showed the only presence of ^{55}Co (Fig. 3).

3.2 Radiolabeling of vancomycin

As long as ethanol was used as the eluent, the radiolabeled compound could not be eluted and remained at the origin. It is not clear why the radiolabeled species are more polar. However, repetition of this procedure yielded the same results (Fig.4). In order to obtain the best labeling reaction conditions, the complex formation was optimized for temperature, time, and amount of vancomycin in the physiologic pH range (5.5~7.0). At room temperature, neutral pH

yielded a successful labeling. Increasing the amount of vancomycin per unit activity from 120 to 250 nanomoles for 185 MBq, enhanced the radiochemical yield up to 96%. Using 140~170 nanomoles of vancomycin was sufficient to achieve a radiolabeling yield higher than 90%.

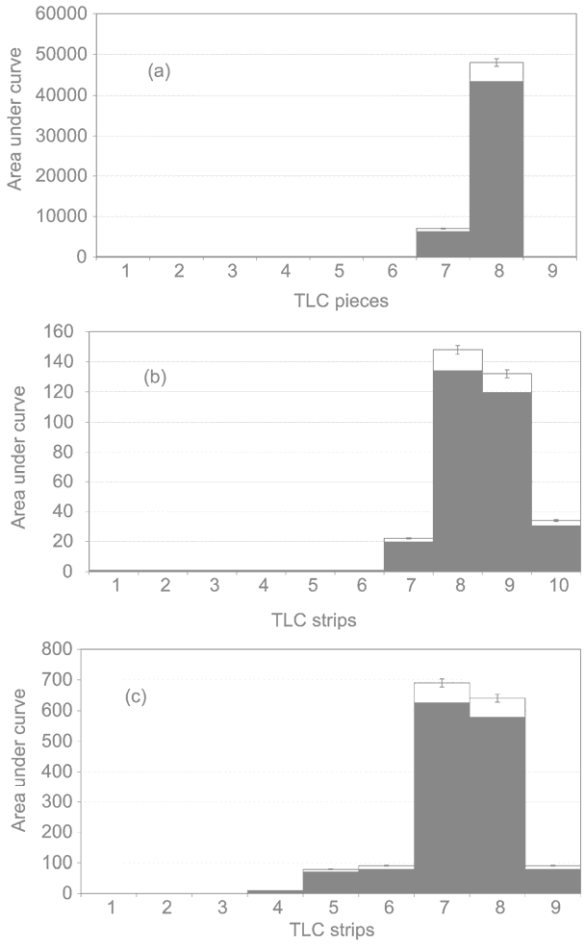

Fig. 3 RTLC of the ^{55}Co sample solutions. (a) [^{55}Co]chloride (n=5) eluted by ethanol as the mobile phase on Si papers, (b) the final [^{55}Co]$CoCl_2$ solution using acetate buffer (pH=7) as the eluent on Si paper, and (c) the final [^{55}Co]$CoCl_2$ solution in normal saline (pH=7) as the eluent on Si paper.

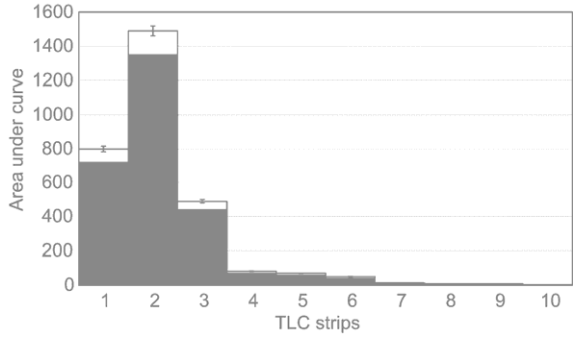

Fig. 4 RTLC of the final [^{55}Co]VAN solution with ethanol as the eluent.

All reactions were performed by a solid phase extraction method using a Si Sep-Pak unit. After loading the column, it was eluted by normal saline. Fractions 1~3 contained no significant activity, while fraction 4 showed the presence of small amounts of labeled compound that was negligible. In fraction 5 however, the best radiochemical yield was obtained. Fraction 6 was again a mixture of free cobalt and the labeled compound (Fig. 5).

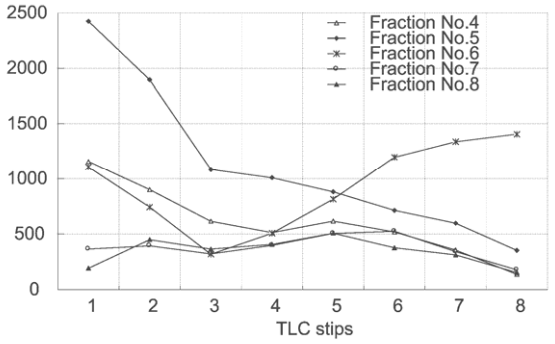

Fig.5 RTLCs of fractions 4~8 of Co-vancomycin elution through Si SepPak using normal saline.

Heating the mixture to 90°C did not increase the yield. Higher temperatures reduced the radiochemical yield due to the decomposition of vancomycin and/or product, resulting in some decomposition products with visible wavelength color (yellow). Thus, room temperature was considered best as it was more facile. At room temperature the radiolabeling was completed in 30 min, shaking the mixture at 10 min-intervals.

[^{55}Co]VAN preparation was sterilized using 0.22 micron filtration. The chemical stability of the final product lasted for 21 h post labeling, suitable to perform further studies. The RTLC of the final product showed no change in stability of [^{55}Co]VAN. Microbial-fungal tests showed a suitable pharmaceutical sterility.

The final product:human serum mixture was incubated at 37°C for 24 h, and samples underwent RTLC tests to study the complex integrity. No change in stability was observed in the first 21 h and the patterns for [^{55}Co]Co^{3+} and [^{55}Co]VAN did not change.

3.3 Biodistribution studies in normal rats

An hour post-injection, the activity appeared in the kidneys and remained high even after 72 h (Fig. 6).

It was shown that during the first 24 h, about 75% of the administered dose of vancomycin was excreted in urine by glomerular filtration. This explains the high accumulation of the tracer in the kidneys [27]. The activity found in brain is low at almost all time points. It has been reported [28] that vancomycin does not readily diffuse across normal meninges into the spinal fluid.

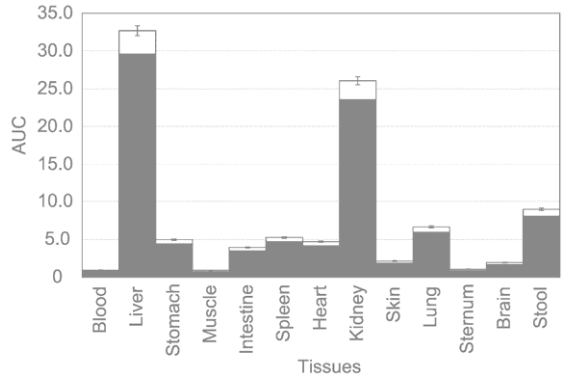

Fig.6 Biodistribution of [^{57}Co]VAN in normal rats 72 h post-injection. AUC: area under curve of the 477.2 keV peak in gamma spectrum.

4 Conclusion

Optimization studies on the production of $^{57}Co^{2+}$ and $^{55}Co^{2+}$ are addressed in this report using a natural nickel target at 22 and 15 MeV proton energies respectively. Total labeling and formulation of [^{55}Co]VAN took about 35 min, with a yield of >90%. A suitable specific activity product was formed *via* insertion of [^{55}Co](II)cobalt cation. The final sample was checked for radiochemical and chemical purity using RTLC. The radio-labeled complex was stable in aqueous solutions for at least 24 days after labeling. No detectable amounts of free [^{55}Co] (II) cobalt (<1%) were detected by RTLC. The stability of the complex was checked in presence of freshly prepared human serum for up to 21 hours, showing the integrity of the complex. The biodistribution of the complex was studied in normal rats, showing the general pattern of vancomycin core, with excretion through kidney in the first couple of hours. This pattern was also observed after IV injection to the normal rats at similar time intervals. [^{55}Co]VAN is a promising PET radiotracer for the future infection imaging.

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