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ORIGINAL PAPER

Production of [^{103}Pd]Bleomycin complex for targeted therapy

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Abstract Due to the anticancer properties of bleomycin (BLM) complexes, production of [^{103}Pd]bleomycin ([^{103}Pd]BLM) was targeted. Palladium-103 ($T_{1/2}$ = 16.96 d) was produced via the ^{103}Rh(p,n)^{103}Pd nuclear reaction using a natural rhodium target. Proton energy was 18 MeV with 200 μA irradiation for 15 h (final activity 25.9 GBq of $^{103}Pd^{2+}$, RCP > 95%, radionuclidic purity > 99%). ^{103}Pd was separated from the irradiated target by anion exchange using a Dowex 1×8 (Cl^-)/100–200 mesh resin in the form of $Pd(NH_3)_2Cl_2$ in order to react with bleomycin to yield [^{103}Pd]BLM. Chemical purity of the final product was in accordance to the accepted limits. [^{103}Pd]BLM was prepared with a radiochemical yield of more than 98% at 80°C in 30 min. The labeling reaction was optimized for time, temperature and ligand concentration. Radiochemical purity of more than 99% was obtained using RTLC with specific activity of about 370 MBq/mmol. The stability of the tracer was checked in the final product and presence of human serum at up to 3 h. The complex was stable in human serum at 37°C up to 2 h of incubation. Biodistribution studies using a SPECT system performed in normal rats in the first 2–3 h.

Key words palladium-103 • palladium(II) complexes • radiolabeling • bleomycin complexes • radiopharmaceuticals • cyclotron • targeted therapy

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Introduction

Palladium-103 has been reported in the literature to be a promising nuclide for radiotherapy in the form of seeds for permanent interstitial implants [10, 14]. The chemical stability of most Pd(II) complexes is well recognized, and the X-ray (20–22 keV) and abundant emission of Auger electrons and half-life (16.96 d) make ^{103}Pd attractive from the dosimetry point of view. Cyclotron based methods have been developed for the production of moderate quantities of carrier-free added ^{103}Pd via the ^{103}Rh(p,n)^{103}Pd reaction [16]. According to our knowledge, Pd-103 labeled compounds are rarely cited in the literature. These factors led us to the study of a number of potential ligands for tumor targeting.

Bleomycin (BLM) is a series of glycoprote in antibiotics isolated from the culture media of *Streptomyces verticillus* [19]. It is already shown that Pd-bleomycin retains anticancer effects and its effect is comparable to that of other metalobleomycin complexes [1]. The BLM used clinically is a mixture of three distinct isomers, A_2 (the most abundant, ≈ 65%), B_2 (≈ 30%) and DM (≈ 5%). Bleomycin is known to form complexes with a range of metals, some of which have been shown to exhibit tumor-localising properties as demonstrated by radiotracer experiments [3], and subsequently by clinical imaging studies [8]. Furthermore, it has been proposed that bleomycin complexes

may be useful for targeted therapy using isotopes of indium [9], ruthenium [15] or rhodium [2].

Based on our previous work on the development of some radiometal imaging tracers [6, 7], we were interested in the development and evaluation of ([^{103}Pd]BLM) complex as a possible therapeutic radiopharmaceutical. Low irradiation range of Pd-103 and DNA attachment of bleomycin as an interchelating antibiotic, provides a selective tumor targeting radiopharmaceutical with specific irradiation on cell dimensions.

Experimental

Materials

Production of ^{103}Pd was performed in the NRCAM (Nuclear Research Center for Agriculture and Medicine) 30 MeV cyclotron (Cyclone-30, IBA). Natural rhodium chloride with a purity of more than 99.9% was supplied by Merck Chemical Company (Germany). Bleomycin sulfate (BLEO-S) was a pharmaceutical sample purchased from Nippon Kayaku Laboratories (Japan). Ammonium acetate and methanol were purchased from Aldrich Chemical Co. (Germany). Analytical HPLC to determine the radiochemical purity was performed by a Shimadzu LC-10AT, equipped with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Si Kromasil 100, 5 μm 250 × 4.6 mm (M & W), Inchrom. A mixture of 10% ammonium acetate:methanol (50:50) was used as eluent at a flow rate of 2 mL/min. Thin-layer chromatography (TLC) was performed on polymer-backed silica gel (F 1500/LS 254, 20 × 20 cm, TLC Ready Foil, Schleicher & Schuell®, Germany). Normal saline used for labeling was of high purity and had been filtered through 0.22 μ Cativex filters. The distribution of radioactivity along the chromatograms was performed by cutting 5-mm portions of the strip followed by counting with a Canberra™ high purity germanium (HPGe) detector (model GC1020-7500SL). Radionuclidic purity was checked with the same detector. All calculations and RTLC counting were based on the 357 keV peak.

Methods

Production of palladium-103 in [^{103}Pd]Pd(NH$_3$)$_2$Cl$_2$ form

The production of Pd-103 in our Center has been published recently [13]. Briefly, an electroplated natural rhodium target on a copper backing plate was irradiated at an angle of 6 degrees towards the proton beam in order to achieve higher production yield. The separation of Pd-103 from the solubilized rhodium matrix was performed by anion exchange. In the first step, the target was dissolved in 6 N HCl and the Cu/Rh/Pd separation was achieved using a Dowex 1×8 (Cl^-)/100–200 mesh column (1.5 × 10 cm). Copper was removed with 0.03 M HCl. Rhodium was eluted with 6 M HCl and kept for recovery. Finally radio-palladium was eluted with a 1:1 mixture of 0.5 M NH_3/NH_4Cl in the form of [^{103}Pd]Pd(NH$_3$)$_2$Cl$_2$. The resulting high-purity [^{103}Pd]Pd(NH$_3$)$_2$Cl$_2$ solution was used directly in the labeling step.

Chemical purity control

The presence of Cu^{2+} was checked by colorimetric assays. The serial diluted copper standard solutions were checked up on our polarography apparatus limit of detection. For colorimetric assay, our limit of detection was 0.5 ppm. Standard copper concentrations were complexed by dithizone forming a pinkish complex [21].

Labeling of bleomycin with [^{103}Pd]Pd(NH$_3$)$_2$Cl$_2$

[^{103}Pd]Pd(NH$_3$)$_2$Cl$_2$ (2.0×10^8 Bq) dissolved in the alkaline medium obtained above (0.5–2 ml) was transferred in to a 2 ml-vial. The mixture was evaporated by slight warming under a nitrogen flow. A mixture of BLM (0.25 mg) in normal saline (50 μl) was then added and heated at different temperatures (25, 50, 80 or 100°C) and cooled in an ice bath. The active solution was checked for radiochemical purity by polymer-backed silica gel layer chromatography as mentioned in the materials. The final solution was then passed through a 0.22 μm filter and pH was adjusted to 5.5–7 by the addition of 0.1 M sodium acetate buffer.

Radiochemical purity

Radio thin-layer chromatography was performed using a mixture of ammonium acetate and 10% methanol (1:1) as eluent. The radiochromatogram showed two major and distinct peaks at the R_f of 0.40 and 0.70. 0.5 cm piece of the TLC paper was cut carefully and then counted as close as possible to detector surface using 357 KeV as the reference peak. Uncomplexed ^{103}Pd remained at the origin ($R_f = 0.0$).

Stability of [^{103}Pd]BLM complex in the final product

Stability studies were based on the previous studies performed for other radiolabeled bleomycins [5]. A sample of [^{103}Pd]BLM (1.85×10^7 Bq) was kept at room temperature for 50 h and its radiochemical purity was checked by RTLC every 5 h.

Serum stability studies

To 36.1×10^6 Bq of [^{103}Pd]BLM was added 500 μL of freshly prepared human serum. The resulting mixture was incubated at 37°C, and 1.5-μL aliquots were analyzed by radio-TLC after 1, 3, 6 h of incubation to determine the stability of the complex.

Imaging of [^{103}Pd]BLM in normal rats

Normal rats were used for preliminary imaging of ^{103}Pd (reconstituted in normal saline after mild evaporation of the purified Pd solution under a flow of N_2 at 40–50°C) and [^{103}Pd]BLM. Images were taken 3 h after administration of the radiopharmaceutical (3.7×10^6 Bq

in 50 µl of normal saline) by a Dual-Head SPECT system (SMV, France, Sopha DST-XL). The rat-to-high energy septa distance was 12 cm. The useful field of view (UFOV) was 540 mm × 400 mm. The spatial resolution was 18 mm FWHM at the CFOV. Sixty four projections were acquired for 30 s per view with a 64 × 64 matrix.

Results

Radioisotope production

^{103}Pd was prepared by 18 MeV proton bombardment of the ^{nat}Rh target. The target was bombarded with a current intensity of 200 µA for 15 h (3000 µAh). The chemical separation process was based on a no-carrier-added method. The resulting activity of ^{103}Pd was 2.53×10^{10} Bq at the end of bombardment (EOB) and the production yield was 8.44×10^{6} Bq/mAh. Radiochemical separation was performed by an ion exchange chromatography method with a yield of more than 95%. Post-purification radionuclidic control showed the presence of 39.7 (0.068%), 294.9 (0.0028%), 357.5 (0.022%) and 497.1 (0.004%) keV gamma energies, all originating from ^{103}Pd (Fig. 1) and showed a radionuclidic purity higher than 99%.

Chemical purity

The colorimetric assays demonstrated that the copper cation concentration was below 0.5 ppm. This result is below the internationally accepted levels, i.e. 5 ppm [21].

Radiolabeling

The radiolabeling of bleomycins has been extensively reported in the literature. In-111 labeled bleomycin has been reported as a therapeutic agent [9]. Using In-111, the complex formation is fast enough to be done even at room temperature for couple of minutes [18].

In this case, the complex is formed at pH = 5–7. The stability of these complexes was later questionable due to the release of free In-111. In other studies, radio-

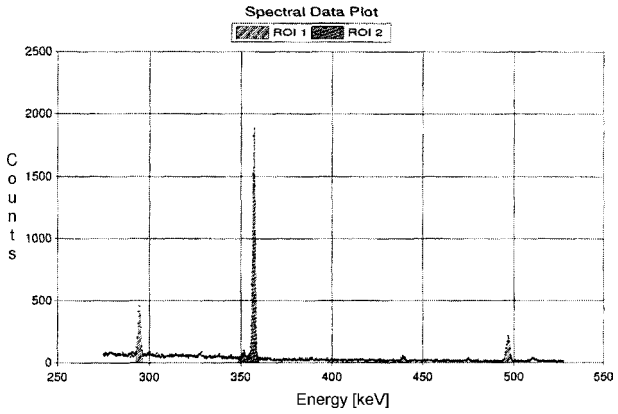

Fig. 1. Gamma spectrum of the final purified [^{103}Pd]Pd$(NH_3)_2Cl_2$ solution.

zinc and copper complexes were formed at higher temperature (about 80 to 100°C) without pH considerations [11].

Due to the X-ray-emitting property of ^{103}Pd and the selective physical properties of this radioisotope, the strategy of incorporating such an isotope with an important antineoplasm compound, i.e. bleomycin, into one moiety was of great interest. In order to obtain the best labeling reaction conditions, the complex formation was optimized for pH, temperature, time, and the amount of bleomycin. At a temperature of 80°C, for instance, the best pH for the labeling step was 5–7, while at higher pHs (7–8) the radiochemical yield was increasing again due to the formation of different labeled species as well as formation of precipitates. In basic conditions, the radiochemical yield decreased drastically due to the degradation of bleomycin to less soluble compounds [17]. At the optimum reaction pH, the yield reached a maximum within 30 min, and stayed constant for longer reaction times. Increasing the ratio of bleomycin to radioactivity increased the labeling yield, presumably due to more available chelate in solution (Fig. 2).

Heating the reaction mixture to 80–90°C increased the yield, which remained constant for temperatures up to 100°C. Further heating reduced the radiochemical yield due to the decomposition of bleomycin and/or product (Fig. 3).

Radiochemical purity

Because of the several polar functional groups in its structure, labeling of bleomycin with a cation does not greatly affect its chromatographic properties. Thus, the

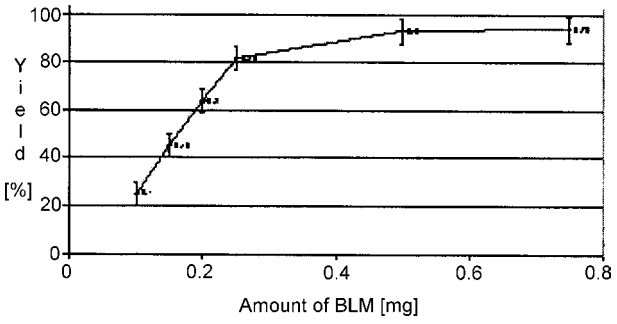

Fig. 2. Effect of BLM amount (mg) on [^{103}Pd]BLM radiochemical yield at an experimental temperature of 80°C at pH 2 (n = 5).

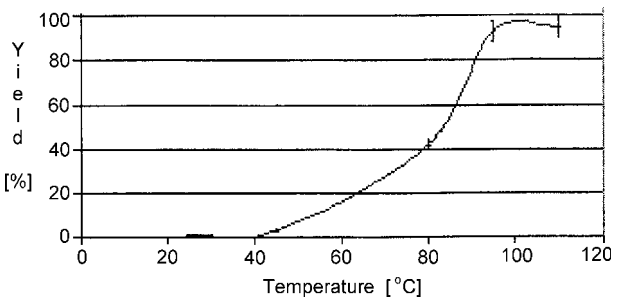

Fig. 3. Effect of temperature on radiochemical yield of [^{103}Pd]BLM in optimized conditions (n = 5).

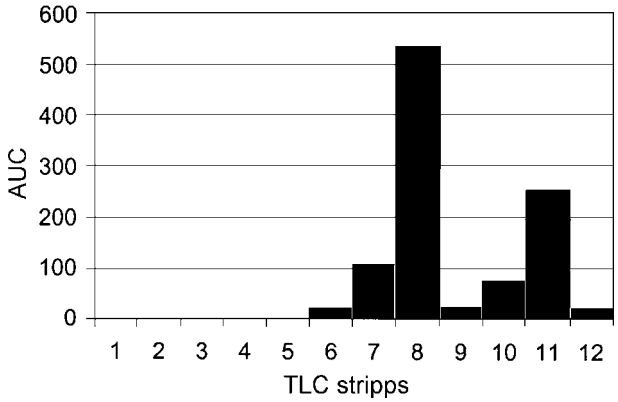

Fig. 4. RTLC of [^{103}Pd]bleomycin sample in optimized conditions.

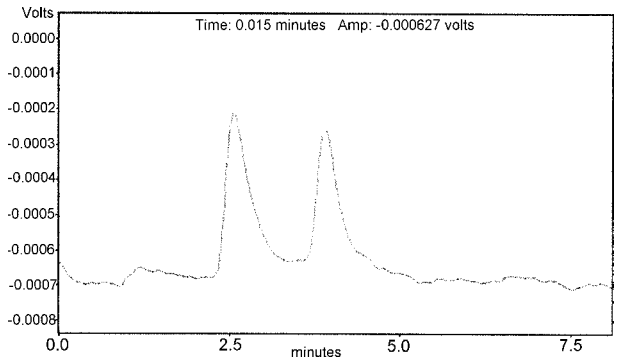

Fig. 5. HPLC chromatogram of pharmaceutical bleomycin sample used in the radiolabeling, flow rate; 2 ml/min, 10% ammonium acetate:methanol (1:1), Si Kromasil, UV; 254 nm.

labeled and unlabeled bleomycin almost migrate to the same R_f. The more polar bleomycin fraction, i.e. bleomycin A_2 correlates to the smallest R_f, while the other polar fractions come at the close R_fs (bleomycin B_2). Since it has been shown that all bleomycins have tumor-seeking properties, separation of the above labeled species was not intended. In all radiolabeling procedures ($n = 5$), the integral ratio of the two peaks were constant (0.7:0.3), showing the isomeric ratio of the two bleomycin chromatogram peaks (Fig. 4). The HPLC chromatogram of the pharmaceutical sample used in the radiolabeling is shown in Fig. 5.

Formulations

Due to its thermal stability, [^{103}Pd]BLM preparation can be autoclaved, but due to the possible loss of palladium from the complex, 0.22 micron filtration was used instead of autoclaving.

Imaging of [^{103}Pd]BLM in normal rats

[^{103}Pd]BLM imaging in the normal rats (not shown) showed a similar accumulation of the free Pd cation in animal organs 2 h post injection, this phenomenon was then confirmed by biological stability studies. A relatively significant amount of activity was accumulated in the bladder showing the excretion of the cation through urinary tract.

Stability tests

The integrity of the complex was showed to be satisfactory up to 50 h, but when challenged with freshly prepared human serum at 37°C stability (higher than 95%) was observed up to 2 h. When tested after 4 h, only 20–30% of the intact complex was detected in the RTLC method.

Discussion

Bleomycin produces suitable and stable complexes with cations like Mg^{2+}, Ca^{2+}, Fe^{2+}, In^{3+} (Fig. 6) [20]. It is believed that bleomycin antibiotics interfere with DNA as false nucleotides' assuming the dithiazole moiety acts like a purine base [4]. On the other hand, these compounds are activated by a cation insertion as anti-neoplasm agents. The whole complex can then act like a peroxidase system, by producing hydrogen peroxide, resulting in DNA decomposition.

The interaction of Pd with bleomycin has been investigated formerly by means of circular dichroism and fluorescence spectroscopy [1]. It has been shown that Pd ion forms equimolar complex with BLM in a three-stage process, but no effort has been made to characterize the different species [12].

Total labeling and formulation of [^{103}Pd]BLM took about 60 min, with a yield of 97%. A suitable specific activity product was formed via insertion of [^{103}Pd]palladium cation. No unlabeled and/or labeled by-products were observed upon RTLC analysis of the final preparations. The radiolabeled complex was stable

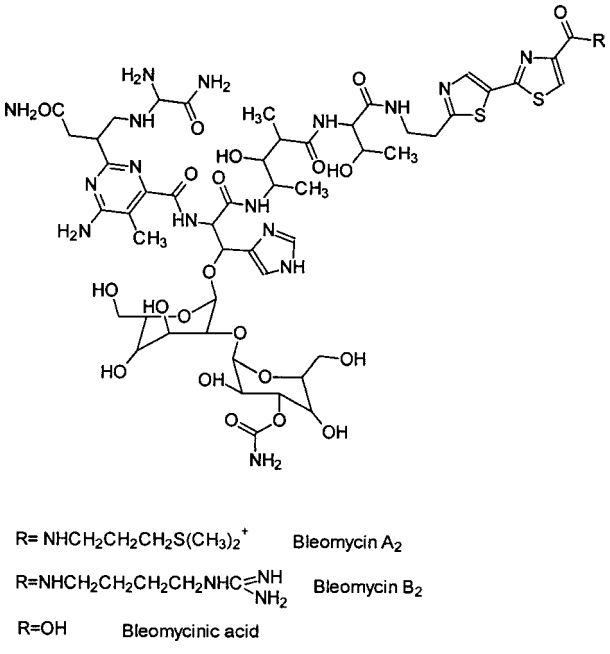

Fig. 6. Structures of bleomycin components in the pharmaceutical sample used.

in aqueous solutions for at least 50 h after labeling. No detectable amounts of free [^{103}Pd]palladium (< 2%) were detected by TLC. RTLC showed that radiochemical purity of the [^{103}Pd]labeled components was higher than 98%. In contrast to other labeled bleomycins, [^{103}Pd]bleomycin, is a therapeutic radiotracer with a long half-life, and the high chemical stability of this radiopharmaceutical could make it a suitable candidate as a therapeutic agent, however due to biological instability of this complex other radiolabeling modalities such as BLM conjugation with polyamine bi-functional chelates must be checked.

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