

Radiosynthesis and evaluation of ytterbium-175 labeled bleomycin as therapeutic agent

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ABSTRACT

Introduction: Bleomycins are DNA-binding biomolecules, which can be used as targeted therapy carriers when labeled with particle-emitters such as Yb-175. In this work the development of Yb-175 bleomycin (¹⁷⁵Yb-BLM) has been reported.

Methods: Yb-175 chloride was obtained by thermal neutron irradiation (3×10^{13} n.cm⁻².s⁻¹) of natural Yb₂O₃ samples at various neutron fluxes and irradiation times. The radionuclide dissolved in acidic media (120mCi/mg) was used in the bleomycin (5 mg) labeling in buffer solution and warming at 60°C for 48 h. Radiochemical purity was determined by ITLC as well as specific activity calculation followed by stability studies. Biodistribution studies of free Yb-175 and ¹⁷⁵Yb-BLM were performed in wild-type mice up to 8 days.

Results: At optimized conditions radiochemical purity of 97±0.88 % and specific activity of 1360 MBq/mM was obtained. Biodistribution studies of free Yb-175 demonstrated liver and bone uptake while in case of ¹⁷⁵Yb-BLM the target tissues were lung, liver and spleen.

Conclusion: ¹⁷⁵Yb-BLM complex was prepared at the optimized conditions and suitable characteristics. The accumulation of the radiolabeled compound in lungs, liver and spleen demonstrates a similar pattern to the other radiolabeled bleomycins. Further studies are to be performed for application of this labeled compound in tumor-bearing models.

Key words: Yb-175; Bleomycin; Quality control; Biodistribution

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INTRODUCTION

Bleomycins (BLMs) are DNA-binding antibiotics produced by *Streptomyces verticillus*. Bleomycin is among non-bone marrow depressive agents widely used in cancer chemotherapy (Figure 1). Bleomycin antibiotics interfere with DNA as false nucleotides, assuming the dithiazole moiety acts like a purine base followed by activation by cation insertion as anti-neoplastic agents with increased bioactivity. It is believed that the whole complex can then act like a peroxidase system, by producing hydrogen peroxide, resulting in DNA decomposition [1]. Based on nucleus targeting properties of BLMs, several radioisotopes have been used in radiolabeling of bleomycins for therapy of neoplastic tissues such as ^{166}Ho [2], ^{103}Ru [3], ^{177}Lu [4] and ^{105}Rh [5].

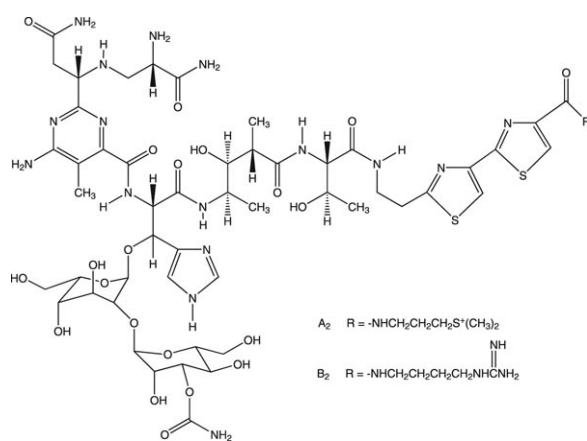


Fig 1. Structure of BLM.

Few Yb-175 radiolabeled complexes are available for therapeutic purposes at research level for palliative therapy of bone metastases [6], radiation synovectomy of small-sized joints in hydroxyl apatite form [7], however no attempts have been made to prepare radiolabeled anti-neoplastic ^{175}Yb -complexes for in vivo studies, according to the authors' knowledge.

^{175}Yb is one of the potential lanthanides with suitable radionuclidic properties for developing various radiotherapeutic agents. ^{175}Yb decays by emission of β -particles with 470 keV maximum energy (86.5%) to stable ^{175}Lu with a convenient half-life of 4.2 days. ^{175}Yb also emits photons of 113 keV (1.9%), 282 keV (3.1%) and 396 keV (6.5%) which are appropriate for studying the biolocalization [8]. ^{175}Yb can be produced by thermal neutron bombardment of natural ytterbium target. The simplified production scheme is: $^{174}\text{Yb} (n, \gamma) ^{175}\text{Yb} \rightarrow ^{175}\text{Lu}$ (Stable) $\sigma = 69$ barn [9] (Table 1).

Table 1: beta decay characteristics for Yb-175 radionuclide [8].

Maximum Energy of beta (keV)	Abundance (%)
73.67	10.2
356.2	3.3
470	86.5

Radiolabeling of bleomycins with various metals and radiometals have been described since 1980s, it is believed that at least 4 nitrogen groups at the polypeptide part are responsible for labeling. In continuation of our research projects for development of new radiopharmaceuticals using reactor produced beta-emitters [10], we described to develop a new target at the cellular level. Due to the interesting pharmacological properties of bleomycins such as solubility in serum, rapid wash-out, tumor avidity and feasible complexation with various bi/tri-valent metals [11], the idea of developing a possible therapeutic agent using beta-emitters by incorporating ^{175}Yb into BLM molecule was investigated. In this work we report, synthesis, radiolabeling, quality control, stability and biodistribution studies of ^{175}Yb -BLM in wild-type mice.

METHODS

The natural ytterbium oxide was purchased from Isotec Inc, USA and ^{175}Yb was produced in the Tehran Research Reactor (TRR). Chemical components were obtained from Sigma-Aldrich Chemical Co. U.K. All radioactivities counting related to paper chromatography were carried out using a NaI (Tl) scintillation counter on adjustment of the base line at 396 keV. The activity as well as the radionuclidic purity of the ^{175}Yb spectroscopy on the base of 396 keV peak by using the HPGe detector and beta spectroscopy was carried out by the Wallac 1220 Quantulus liquid scintillation spectrometer. Bleomycin sulfate (BLEO-S) was a pharmaceutical sample purchased from Nippon Kayaku Laboratories, Japan. Thin layer chromatography (TLC) for cold compounds was performed on polymer-backed silica gel (F 1500/LS 254, 20 9 20 cm, TLC Ready Foil, Schleicher & Schuell, Germany). Normal saline and sodium acetate used for labeling were of high purity and had been filtered through 0.22 μm Cativex filters. Instant thin layer chromatography (ITLC) was performed by counting Whatman No. 2 papers using a thin layer chromatography scanner, Bioscan AR2000, Bioscan Europe Ltd. (France). For activity measurement of the samples a CRC Capintech Radiometer (NJ, USA) was used. All calculations and tissue counting were based on the 399 keV peak.

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.

Production and quality control of $^{175}\text{YbCl}_3$ solution

Ytterbium-175 was produced by neutron irradiation of 1 mg of natural Yb_2O_3 at the neutron flux of 3×10^{13} n/cm²/s. Irradiation was carried out for 7 d. The irradiated target was dissolved in 0.1 M HCl and the resultant solution was evaporated and was reconstituted in double distilled water. The radionuclidic purity of the solution was checked using high purity germanium (HPGe) spectroscopy for the detection of various interfering gamma emitting radionuclides and also beta scintillation tests.

Quality control of the product

Radionuclidic purity: Gamma spectroscopy of the final sample was carried out counting in an HPGe detector coupled to a Canberra TM multi-channel analyzer for 1000 s.

Radiochemical purity control: The radiochemical purity of the $^{175}\text{YbCl}_3$ was checked using two solvent systems by ITLC on Whatman No. 2 papers. A: 10% NH_4OAc and methanol (1:1) and B: 10mM DTPA solution (pH 5).

Preparation of ^{175}Yb -BLM

Radiolabeling of bleomycin using $^{175}\text{Yb}^{3+}$ solution was performed according to previously reported methods [12]. Briefly, 1.1 mCi of $^{175}\text{YbCl}_3$ (40.7GBq) in acidic medium was transferred to a 2 ml vial and evaporated by slight warming under a nitrogen flow. Then, BLM aqueous stock solution (5mg in 1.5 ml) and phosphate buffer solution (0.5 ml) was added to activity containing vial. The mixture stirred and heated at 50°C for up to 48 h. The active solution checked for radiochemical purity by spotting 5 μl sample of the final fraction on a chromatography Whatman No. 2 paper, developed in mobile phase mixture, 10% NH_4OAc and methanol 1:1. After completion, the pH of final solution was adjusted to 5.5–7 by addition of 1 mol/L sodium acetate buffer followed by sterile filtration.

Stability tests

In final solution: The stability of the complex was checked according to the conventional ITLC method [13]. A sample of [^{175}Yb]-BLM (37 MBq) was kept at room temperature for 2 days while being checked by ITLC at time intervals in order to check stability in final product using above chromatography system.

In presence of human serum: For serum stability studies, 300 μL of freshly prepared healthy human serum was added to 200 μCi (100 μL) of ^{175}Yb -BLM final solution and the resulting mixture was incubated at 37°C for 48 h. Every 12 h a portion of the 50 μL of the mixture, trichloroacetic acid (10%, 100 μL) was added and the mixture was centrifuged at 3000 rpm for 5 min followed by decanting the supernatant from the debris. The stability was determined by RTLC analysis of supernatant using above mentioned TLC system.

Biodistribution in wild-type mice

The distribution of the radiolabeled complex as well as free ytterbium-175 cation among tissues was determined for wild-type mice. The total amount of radioactivity injected into each animal was measured by counting the 1-ml syringe before and after injection in a dose calibrator with fixed geometry. The animals were sacrificed using the animal care protocols at selected times after injection, the tissues (blood, heart, lung, brain, intestine, feces, skin, stomach, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline and their specific activities were determined with an NaI detector equipped with a sample holder device as percent of injected dose per gram of tissues. Blood samples were rapidly taken from rodent aorta after scarification.

RESULTS AND DISCUSSION

Production of ^{175}Yb

Around 1.3-1.5 GBq/g (35-40 Ci/g) of ^{175}Yb activity was obtained after 7 days irradiation at a flux of 3×10^{13} n/cm²/s using natural Yb_2O_3 target. A thorough study on the various irradiation times and neutron fluxes performed for radionuclide production leading to the optimized condition determination. Major radionuclidic impurities in irradiated samples were shown to be ^{169}Yb and ^{177}Lu based on time of irradiation.

The observed gamma-photo peaks correspond to the gamma-photo peaks of ^{175}Yb (113, 144, 286 and 396 keV), ^{169}Yb (63, 110, 130, 177, 198, 261 and 307 KeV) and ^{177}Lu (208 and 250 keV). By analyzing the gamma-ray spectra, the radionuclidic purity of ^{175}Yb was found to be 96.2% with the presence of 2.1% ^{169}Yb . Beta spectrum of the final sample used in radiolabeling was also obtained showing a maximum peak around channel 450.

For radiochemical purity two solvent systems were used. In 10% NH_4OAc :methanol mixture (1:1) mixture the free cation remains at the base while any undistinguished anions would migrate to higher R_fs (not observed). On the other hand in 10 mM

DTPA solution Yb-^{175} cation is complexed in Yb-DTPA form migrating to higher R_f s and any possible colloidal fraction would remain at the base.

Preparation of Yb-^{175} bleomycin

The measurements on the oxidation-reduction potential of various metal-bleomycins suggested that the potentials were within a range that would allow the reduction of metal-bleomycin to take place in a cell [14]. Thus the incorporation of the whole complex into cells is possible, especially at the high thiol levels for many tumor cells containing metallothioproteins [15] while most of metal-BLM complexes are reportedly kinetically and thermodynamically stable in ligand substitution processes and are only slowly reduced and dissociated by sulfhydryl reagents. All these data support the possibility of development of an interesting metal radionuclide BLM complex with therapeutic properties such as Yb-^{175}

Most of bleomycin pharmaceutical samples include various active components with a range of antineoplastic activity however the most dominant fractions are BLM- A_2 and BLM- B_2 as compared to the bleomycin mixture. The other fractions including BLM- A_1 and demethylated BLM- A_2 possess lower tumor uptake but present in the samples [16]. Because of the engagement of NH polar functional groups in its structure, labeling of BLM with ytterbium cation affects its chromatographic properties and the final complex is more lipophilic.

In chromatographic studies, two major components are eluted at 0.57 and 0.82. regarding the cationic properties of the A_2 [$-\text{S-CH}_3^{2+}$], it is assumed that this component is eluted at the lower R_f , however the other dominant peak at 0.82 is related to BLM- B_2 with guanidine moiety, also the A_2/B_2 concentration ratio in pharmaceutical samples are reported to be about 3:1 which are in agreement with the radiochromatogram (Figure 2). Chromatographic system was used for the detection of the radiolabeled compound from the free ytterbium cation. Using 10% NH_4OAc and methanol 1:1 mixture, free ytterbium remains at the origin of the paper as a single peak, while the radiolabeled compounds migrate to higher R_f .

Stability

The chemical stability of [^{175}Yb]-BLM was high enough so that the radiochemical purity of complex remained 98% for 2 days in the final formulation. However, under physiologic conditions, incubation of [^{175}Yb]-BLM in freshly prepared human serum for 2 days at 37°C showed 20-25% loss of ^{175}Yb from the complex still enough for biolocalization in case of tumor studies (Figure 3).

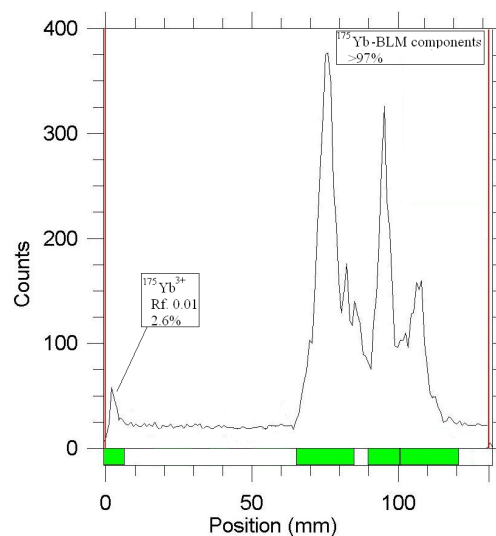


Fig 2. ITLC of ^{175}Yb -BLM in a 10% NH_4OAc and methanol 1:1 mixture as mobile phase on Whatman No.2 papers.

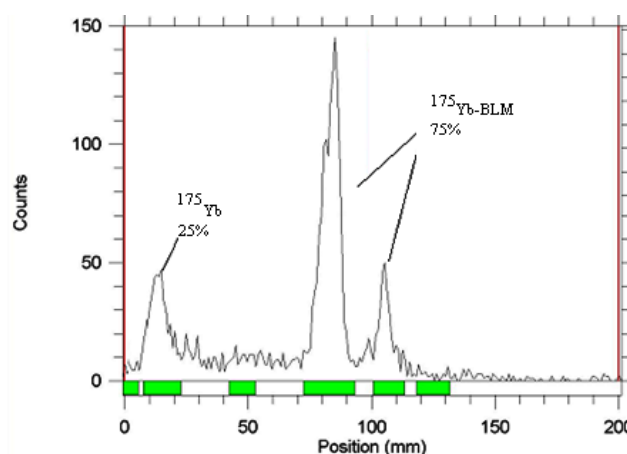


Fig 3. ITLC of ^{175}Yb -BLM solution:serum mixture supernatant at 37°C after 48h in 10% NH_4OAc and methanol 1:1 mixture as mobile phase on Whatman No.2 papers.

Biodistribution

For better comparison biodistribution study was performed for free Yb^{3+} as well. The %ID/g data are summarized in Figure 4. For free Yb^{3+} cation, the radioactivity was mainly located in the liver, kidney and bone. The free cation is soluble in water and it can be excreted *via* the urinary tract. Since the metallic Yb^{3+} is transferred in plasma into a protein-bond form, the major final accumulation was shown to be in the liver reaching >3% after 8 days. Also based on bio-equality of lanthanide cations with calcium ions, the Yb^{3+} is also absorbed on hydroxyl apatite texture of the bone so in 48 hours a 2.25% bone uptake is observed.

However, the biodistribution of ^{175}Yb -BLM demonstrated different pattern as already shown for

other radiolabeled bleomycins including Ho-166 bleomycin.

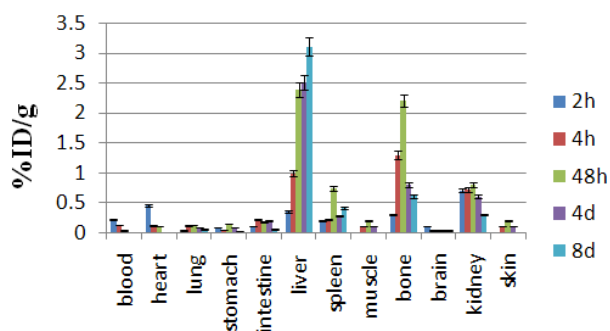


Fig 4. Biodistribution of $^{175}\text{YbCl}_3$ (1.85 MBq, 50 μCi) in wild-type mice 2, 4, 48 h and 4, 8 days after iv injection via tail vein (ID/g%: percentage of injected dose per gram of tissue (n=5)).

The difference is mostly due to the biological stability of Yb-BLM complex compared to Ho-BLM complex, although further investigation should be performed for this suggestion. For instance lung uptake is rather high for the complex. As reported previously a rather severe side effect of bleomycin chemotherapy in lung fibrosis due to high BLM accumulation in this tissue. Liver and spleen are also two other accumulation sites of bleomycin. The hepatobiliary excretion of BLM accounts for 30-40% of the metabolic fate (Figure 5).

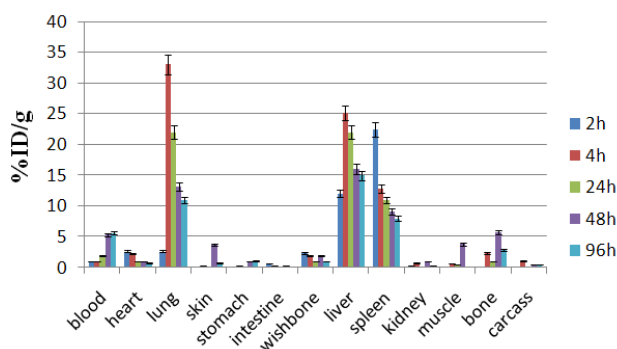


Fig 5. Biodistribution of ^{175}Yb -BLM (1.85 MBq, 50 μCi) in wild type rats 2,4,24,48 and 96 h after iv injection via tail vein (ID/g%:percentage of injected dose per gram of tissue (n= 3)).

CONCLUSION

Total labeling and formulation of ^{175}Yb -BLM took about 48h (RCP >97% ITLC, specific activity: 1360 MBq/mmol). The complex was stable in final formulation and human serum at least for 24 h. The biodistribution of the labeled compound in vital organs of wild-type mice was studied using

sacrification studies and SPECT imaging up to 96 h. A detailed comparative pharmacokinetic study performed for ^{175}Yb cation and ^{175}Yb -BLM. The complex is mostly washed out from the circulation through hepatobiliary system.

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