Preparation and quality control of scandium-46 bleomycin as a possible therapeutic agent

Original Article

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ABSTRACT

Introduction: Due to interesting therapeutic properties of ^{46}Sc and antineoblastic antibiotic, bleomycin (BLM), ^{46}Sc-bleomycin (^{46}Sc-BLM) was developed as a possible therapeutic compound.

Methods: In this work, Sc-46 chloride was obtained by thermal neutron flux (4×10^{13} n.cm^{-2}.s^{-1}) of natural metallic scandium sample followed by dissolution in acidic media as a substitute for ^{47}Sc in radiolabeling studies which was further used for labeling of bleomycin (BLM) followed by stability studies as well as biodistribution in wild-type rats.

Results: Sc-46 was obtained in high radiochemical purity (ITLC, >99%, two systems) as well as acceptable specific activity. At optimized conditions a radiochemical purity of 98% was obtained for ^{46}Sc-BLM shown by ITLC (Specific activity, 740 GBq/mmole). The accumulation of the radiolabeled compound in lungs, liver and spleen demonstrates a similar pattern to the other radiolabeled bleomycins.

Conclusion: Sc-BLM is a possible therapeutic agent in human malignancies and the efficacy of the compound should be tested in various tumor-bearing models.

Key words: Bleomycin, Sc-46, Biodistribution, Radiolabeling

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INTRODUCTION

Scandium is a silvery-white metallic element found in various rare minerals and separated as a by-product in the processing of certain uranium ores. The only naturally occurring isotope is ^{45}Sc (N.A. 100%). Scandium-46 is a beta-emitter radionuclide (mean beta energy of 0.3569 MeV) with 83.8 d half life which emits 2 gamma rays (0.889 and 1.120 MeV) [1].

Among scandium radionuclides, ^{46}Sc and ^{47}Sc have been used in biological and medical studies. Scandium-47 is one example of a beta-emitting isotope with a short half-life of 3.34 days. Because the availability of ^{47}Sc isotope is currently extremely limited, we have performed preliminary studies using instead ^{46}Sc which is chemically identical. A long half-life (83.8 days) and high-energy beta-emissions render ^{46}Sc ideal for assessing the chemistry, stability, and biodistribution of scandium-labeled compounds.

However, its long half-life and emission characteristics are unsuitable for clinical studies. These results suggest the feasibility of future experiments with ^{47}Sc. Scandium-46 has been used in many biological studies in the literature. For instance, high parathyroid uptake of Sc-46 has suggested the use of this radionuclide for tumor therapy and diagnostic studies [2].

Also, ^{46}Sc-labeled microsphere has been vastly used in the determination of blood flow in many cardiovascular studies [3, 4]. In veterinary, suitability of ^{46}Sc as a non-absorbed reference material for nutritional studies has been demonstrated [5].

Bleomycins are tumor seeking antibiotics that are widely used in cancer chemotherapy (Fig. 1).

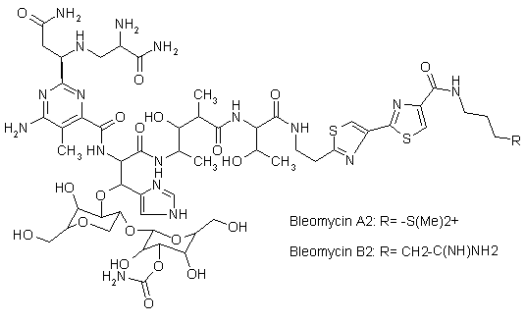

Fig 1. Structures of bleomycin major components in the pharmaceutical sample.

It is believed that bleomycin antibiotics interfere with DNA as false nucleotides, assuming the dithiazole moiety acts like a purine base [6]. It has been shown that labeling of bleomycin with bi/trivalent radioisotopes can produce pharmacologically active compounds carrying a diagnostic and/or therapeutic radioisotope depending on the decay type [7]. The measurements on 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 [8].

Thus the incorporation of the whole complex into cells are possible, especially at the high thiol levels for many tumor cells containing metallothioproteins 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 with therapeutic properties such as scandium-177.

Physicochemical properties of bleomycin trivalent lanthanide ion complexes have been already studied [9]. We have recently reported a significant human breast cancer xenograft uptake for ^{153}Sm-bleomycin complex [10] and ^{177}Lu-bleomycin complexes and the therapeutic studies on the tumor models are underway.Several radioisotopes such as ^{103}Ru [11] and ^{105}Rh [12] have been used in radiolabeling of bleomycins for therapy of neoplastic tissues. In continuation of developing radiolabeled bleomycins using various diagnostic/therapeutic radioisotopes [13, 14], we hereby report preparation, stability tests and biodistribution of ^{46}Sc-BLM as a potential therapeutic complex.

METHODS

^{46}Sc was produced with a specific activity of approximately 7.0-7.5 mCi/mg and radionuclidic purity of >98% by irradiation of natural metallic scandium sample (2 mg) targeted at a thermal neutron flux of approximately 4×10^{13} n/cm^2.s for 3 days at Tehran Research Reactor (TRR), followed by decay period (20 days). Sephadex G-50, sodium acetate, phosphate buffer components, methanol and ammonium acetate were purchased from Sigma-Aldrich Chemical Co. (U.K.). Whatman No. 1 paper was obtained from Whatman (Maidstone, UK) for instant thin layer chromatography (ITLC). Radio-chromatography was performed by using a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in rat organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 889 keV peak for ^{46}Sc. All values were

expressed as mean ± standard deviation (Mean± SD) and the data were compared using student T-test. Statistical significance was defined as $P<0.05$. 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. Male healthy rats were purchased from Pasteur Institute, Tehran, Iran.

Production and quality control of $^{46}ScCl_3$ solution

Scandium-46 was prepared by flux of 4×10^{13} n/cm^2.s. Specific activity of the produced ^{46}Sc was 7.5 mCi/mg after irradiation for 3 days. The irradiated target was dissolved in 200 µl of 1.0 M HCl, to prepare $^{46}ScCl_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution of final volume of 5 ml. The mixture was filtered through a 0.22 µm biological filter and sent for use in the radiolableing step. The radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy as well as HPGe spectroscopy for the detection of various interfering beta and gamma emitting radionuclides. The radiochemical purity of the $^{46}ScCl_3$ was checked using 2 solvent systems for ITLC (A: 10mM DTPA pH.5 aq. solution and B: %10ammonium acetate:methanol (1:1)).

Labeling of bleomycin with $^{46}ScCl_3$

Radiolabeling of bleomycin using cation solution was performed according to previously reported methods [15]. Briefly, $^{46}ScCl_3$ (74-150 MBq) dissolved in 0.5-2 mL of acidic medium (1M HCl) was transferred to a 2 ml-vial and the mixture was evaporated by slight warming under a nitrogen flow. Volumes of BLM aqueous stock solution (3mg/ml) were added to activity containing vials and the total volumes were taken up to 0.5 ml by normal saline addition. The mixtures were stirred at room temperature for up to 48 h. The active solution was checked for radiochemical purity by ITLC every two hour. The final solution was then passed through a 0.22 µ filter and pH was adjusted to 5.5-7 by addition of 1 mol/L sodium acetate buffer.

Quality control of ^{46}Sc-BLM

A 5 µL sample of the final fraction was spotted on chromatography paper Whatman No.2, and developed in a mixture of 10 mmol/L DTPA solution as mobile phase to discriminate free scandium from radiolabeled compound.

Stability of ^{46}Sc-BLM complex in the final product

Stability tests were based on previous studies performed for other radiolabeled bleomycins [16]. A sample of ^{46}Sc-BLM (18-180 MBq) was kept at room temperature for 48 h while checked by RTLC every 4 h. A micropipet sample (5 µL) was taken from the shaking mixture and the ratio of free radio-scandium to ^{46}Sc-BLM was checked by ITLC in a mixture of 10 mmol/L DTPA solution as mobile phase to discriminate free scandium from radiolabeled compound.

Serum stability studies

500µL of freshly prepared human serum was added to 37MBq (100µL) of ^{46}Sc-BLM and the resulting mixture was incubated at 37°C for 24 h. Aliquots (5-µL) were analyzed by ITLC up to 24h of incubation to determine the stability of the complex.

Biodistribution of $^{46}ScCl_3$ and ^{46}Sc-BLM in wild-type rats

$^{46}ScCl_3$ and ^{46}Sc-BLM were administered to separate wild-type rat groups. A volume (50-100 µL) of ^{46}Sc-BLM or $^{46}ScCl_3$ solutions containing radioactivity (5.55 MBq) were injected intravenously *via* their tail veins. The animals were sacrificed at different time intervals (2, 4, 24 and 48 h) for $^{46}ScCl_3$ and 2, 24 and 5d for ^{46}Sc-BLM), and the ID/g % of different organs was calculated as percentage of injected dose (based on area under the curve of 889keV peak) per gram.

RESULTS AND DISCUSSION

Production and quality control of ^{46}Sc

The radionuclidic purity of the product was checked by gamma-ray spectroscopy. Only the two characteristic peaks of ^{46}Sc at 889 and 1120 keV were observed (Fig. 2).

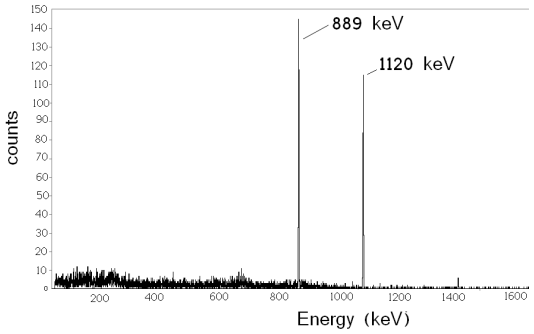

Fig 2. HPGe spectrum of $^{46}ScCl_3$ solution used in this study.

Scandium is monoisotopic with the atomic mass 45 and usually, besides ^{46}Sc, formation of any other scandium radionuclide such as Sc-47 is not expected.

However, ^{45}Ca may be formed via the (n,p) reaction. It is a pure beta emitter and thus difficult to detect. On the other hand considering the cross sections of (n,γ) and (n,p) reactions on 45Sc as well as the half lives of 46Sc and 45Ca, it is estimated that the 45Ca impurity, if at all, was <0.1%.

The radiochemical purity of the ^{46}Sc solution was checked using ITLC and in two solvent systems; in 10 mM DTPA, free Sc^{3+} cation was complexed into more lipophilic Sc-DTPA form and migrated to higher R_f, while almost no radioactive fraction remained at the origin, suggesting the high radiochemical purity of the sample.

The second system was ammonium acetate: methanol mixture. Any fast eluting species would possibly be Sc-46 species other than Sc^{3+} and the remaining fraction at R_f =0.1 would be Sc^{3+}. In both chromatographic systems no other radiochemical species was detected (Fig. 3).

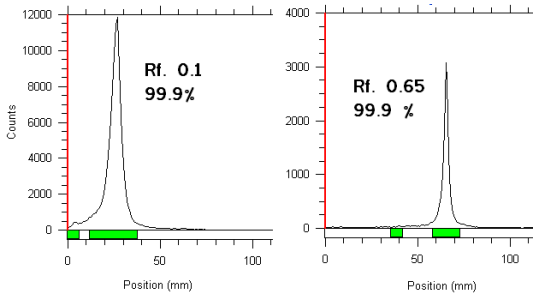

Fig 3. Radio chromatogram of free Sc^{3+} cation in 10% ammonium acetate:methanol mixture (pH.5) (left) and 10 mM DTPA at optimized conditions (right).

Preparation of ^{46}Sc-BLM

In order to obtain the highest specific activity in shortest possible time, a quantitative study was designed using different amounts of BLM and various time intervals for a specific amount of radioactivity (2 mCi of $ScCl_3$ for instance) while 25ºC was considered suitable temperature. A satisfactory labeling yield of 94-97% was obtained at room temperature using 0.15-0.3 mg of BLM within 24-48 h.

Because of relatively high molecular weight and several polar functional groups in its structure, BLM retains at the origin on ITLC. Also radiolabeling of bleomycin with cations does not greatly affect its chromatographic properties. Thus the labeled and unlabeled bleomycins almost remain at the same R_f (0.0) using ITLC. On the other hand, due to the tumor-seeking properties of all bleomycin components in the pharmaceutical sample, separation of the above labeled BLM species was not intended.

As shown in Figure 1 the pharmaceutical sample is majorly composed of 2 components with reported ratio mixture [17], considering the molar ratio, a mean molecular weight of 1495.22 can be calculated, resulting in a specific activity of 740 GBq/mmole at optimized radiolabeling conditions. The labeling step took about 24 h. In all radiolabeling procedures (n=5), the labeling yield was over 99%. The ratio of ^{46}Sc-BLM peaks at R_fs of 0.55 to free Sc^{3+} radiopeak (R_f: 0.1) was considered as the radiochemical yield using solvent system A. (Fig. 4).

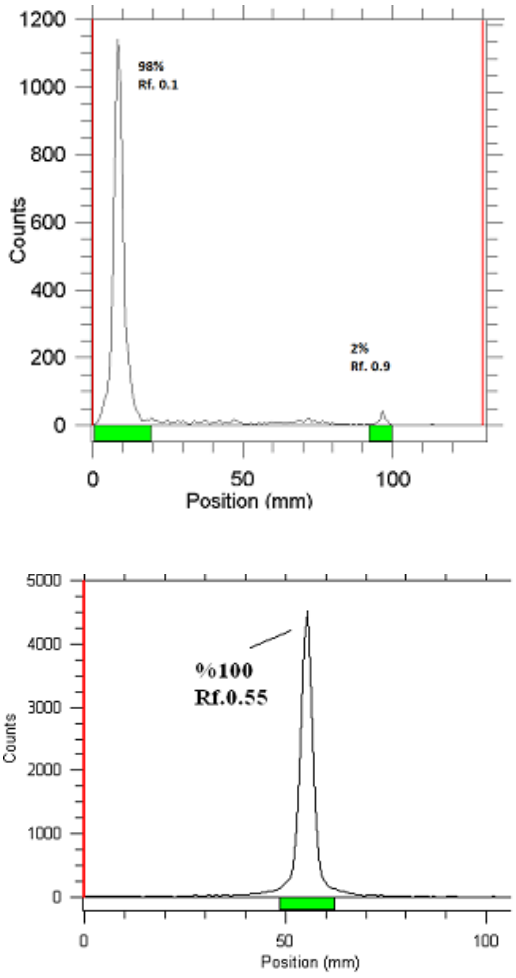

Fig 4. Radio chromatogram of free Sc^{3+} cation (above) and ^{46}Sc-BLM (below) in 10% ammonium acetate: methanol mixture (pH.5) at optimized conditions.

For optimization of the labeling conditions, at room temperature, the best pH for the labeling step was 5.5 -7. At basic conditions the radiochemical yield decreased drastically due to the degradation of bleomycin to less soluble compounds [18].

The final radiolabeled complex diluted in normal saline was then passed through a 0.22 µm (Millipore)

filter for sterilization. Incubation of ^{46}Sc-BLM in freshly prepared human serum for 24 h at 37°C showed no loss of ^{46}Sc from the complex at least for 48 h.

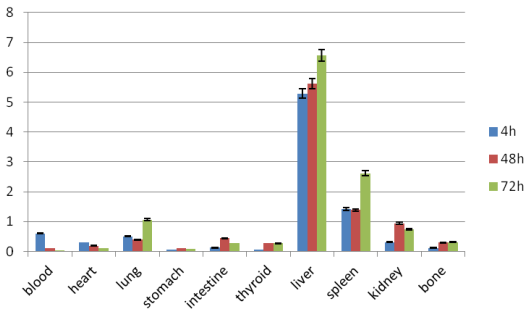

Fig 5. Calculated ID/g in % of i.v. injection of $^{46}ScCl_3$ (40 µCi) in wild-type rats, 4, 48 and 72 hours post injection (n=3).

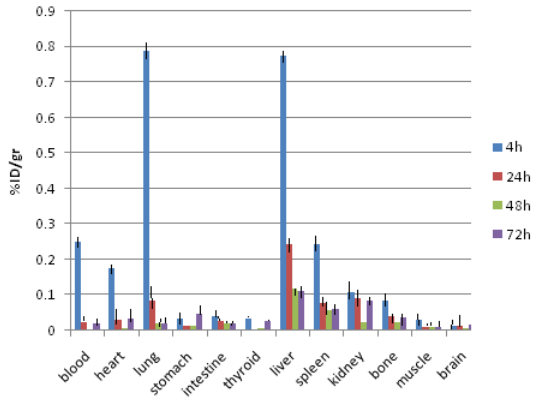

Fig 6. Percentage of injected dose per gram (ID/g %) of ^{46}Sc-BLM in wild-type rat tissues at 4, 24, 48 and 72h post injection.

Biodistribution studies

The pattern for scandium biodistribution among animal tissues was comparable to most of other aluminium metal group members (including Ga, In, etc.). The liver is the major target organ due to the presence of metaloproteins, apart from the liver, the spleen is the second major accumuation target. These results are comparable to the reported data concerning scandium-citrate administered to mice and reported by Ford-Hutchinson et al. [19]. Thus, transferrin is principally responsible for scandium metabolism *in vivo*, while binding with ferritin is negligible [20]. Slight differences in the reported biodistribution data and the present work are possibly because of using rats instead of mice (Fig. 5).

The accumulation of ^{46}Sc-BLM is demonstrated in Figure 6. The lung, liver and kidney were the major accumulation sites of the radiolabeled bleomycins, which have similar biokinetic to free BLMs. A major route of excretion for the tracer was urinary tract similar to BLM.

Comparison of vital organs uptake for $^{46}ScCl_3$ and ^{46}Sc-BLM demonstrates kinetic pattern difference for both species. ^{46}Sc cation is accumulated in the liver in first 24h post injection, and it can be assumed that later the activity is excreted from liver *via* biliary tract, while ^{46}Sc-BLM is excreted through kidneys with an exponential rate in 3 days.

In case of lung almost no detectable activity was accumulated as already shown for most of other radiometals, while ScBLM is majorly deposited in lung due to reported side effects for this antibiotic.

CONCLUSION

Sc-46 was prepared by irradiation of natural metallic scandium sample by 4 x 10^{13} n.sec^{-1}.cm^{-2} (radionuclide purity >98%). The radiochemical purity of the sample was checked by ITLC (>99% using two solvent systems). At optimized conditions, total labeling and formulation of ^{46}Sc-BLM took about 24 h, with a radiochemical yield higher than % 98. The radio-labeled complex was stable in aqueous solutions for at least 48 hours and no significant amount of other radioactive species was detected by ITLC. Trace amounts of $^{46}ScCl_3$ (≈2%) were detected by ITLC. Specific activity calculated for the radiolabeled compound was 740 GBq/mmole. The biodistribution of labeled compound was checked in wild-type rats up to 3 days and a significant accumulation took place in liver, spleen and kidneys which is in accordance with the biodistribution of other reported radiolabeld bleomycin compounds. ^{46}Sc-BLM is a potential therapeutic compound and our experiments on this compound have shown satisfactory quality, and stability suitable for future therapeutic studies.

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