Preparation and quality control of lutetium-I77 bleomycin as a possible therapeutic agent

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Abstract. Due to interesting therapeutic properties of ^{177}Lu and antineoblastic antibiotic, bleomycin (BLM), ^{177}Lu-bleomycin (^{177}Lu-BLM) was developed as a possible therapeutic compound. Lu-177 of 2.6–3 GBq/mg specific activity was obtained by irradiation of a natural Lu_2O_3 sample with a thermal neutron flux of 4×10^{13} n·cm^{-2}·s^{-1}. The product was converted into chloride form which was further used for labeling of BLM. In optimized conditions a radiochemical purity of 98% was obtained for ^{177}Lu-BLM shown by instant thin-layer chromatography (ITLC) (specific activity, 740 GBq/mmole). Biodistribution studies of Lu-177 chloride and ^{177}Lu-BLM were performed in wild-type rats. The accumulation of the radiolabeled compound in lungs, liver and spleen demonstrates a pattern similar to the other radiolabeled bleomycins. Lu-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 • Lu-177 • biodistribution • radiolabeling

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Introduction

Bleomycins are tumor seeking antibiotics that are widely used in cancer chemotherapy (Fig. 1). It is believed that bleomycin antibiotics interfere with DNA as false nucleotides, assuming the dithiazole moiety acts like a purine base [20]. 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 [12].

The measurements of 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 high thiol levels for many tumor cells containing metallothioproteins, while most of the 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 lutetium-177.

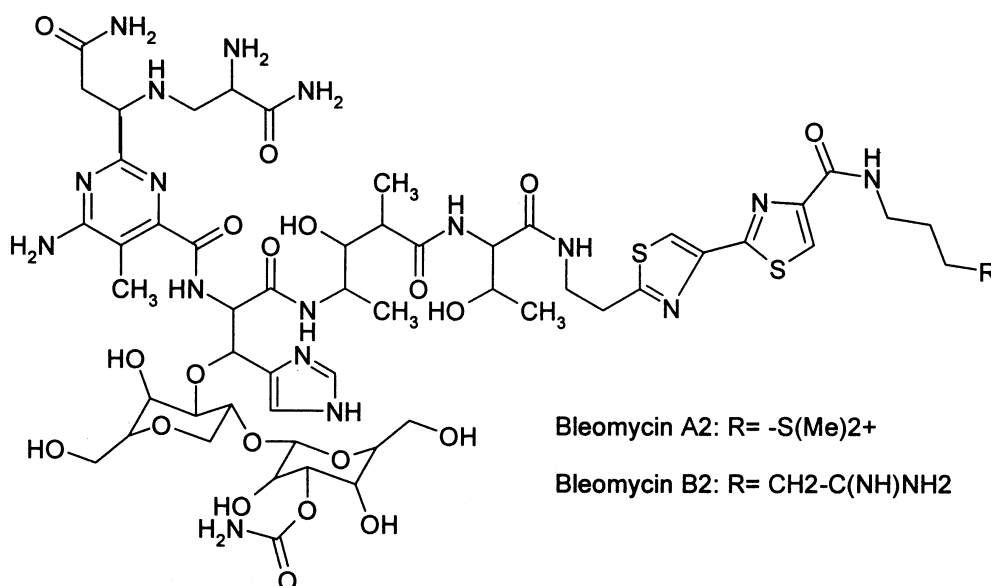Physicochemical properties of bleomycin trivalent lanthanide ion complexes have been already studied

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

[13]. Among lanthanides, terbium has been used in the complexation of bleomycin and the complex was shown to possess luminescence properties which can be used in the detection of the drug in solutions [21]. In one report, [153]Sm(III), [140]La(III) and [169]Yb(III)-bleomycin complexes have been prepared and their distribution in mice bearing sarcoma-180 tumors were studied.

The authors stated that compared to gallium-67, Sm(III), Yb(III), compounds exhibited enhanced localization in tumors with concomitant lower body background and proposed possible clinical uses of BLM lanthanide complexes for tumor diagnosis, however no further attempts were reported for diagnosis and therapeutic studies of these complexes [18]. We have recently reported a significant human breast cancer xenograft uptake for [153]Sm-bleomycin complex [1] and therapeutic studies on the tumor models are underway.

Many β⁻-emitters such as Sm-153, Ho-166 and Lu-177 can be produced in reasonable amounts using (n,γ) reactions. Due to the half-life limitations in the application of the mentioned radionuclides, the emerging need for a long half-life beta emitter such as lutetium-177 is obvious.

Owing to lutetium-177 suitable decay characteristics [$T_{1/2}$ = 6.73 d, $E\beta_{max}$ = 497 keV, $E\gamma$ = 112 keV (6.4%), 208 keV (11%)] as well as the feasibility of large-scale production in adequate specific activity and radionuclidic purity using a moderate flux reactor, ^{177}Lu has been considered as a promising radionuclide for developing therapeutic radiopharmaceuticals.

Thus, various agents have been developed and used in therapy including ^{177}Lu-labeled compounds, such as somatostatin receptor ligands [2], monoclonal antibodies [15], pain palliation compounds [4] and radiosynovectomy agents [5, 6].

Several radioisotopes such as [103]Ru [17] and [105]Rh [3] have been used in radiolabeling of bleomycins for therapy of neoplastic tissues. In continuation of developing radiolabeled bleomycins using various diagnostic/therapeutic radioisotopes [9, 10], we hereby report preparation, stability tests and biodistribution of [177]Lu--BLM as a potential therapeutic complex.

Experimental

Materials

Bleomycin was a pharmaceutical sample purchased from Merck Co., Spain, and was used without further purification. Chromatography paper (Whatman no. 2) was obtained from Whatman (Maidstone, UK). Radiochromatography was performed using a bioscan AR-2000 radio thin-layer chromatography (TLC) scanner instrument (Bioscan, Paris, France). 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 112 keV peak for [177]Lu. All values were expressed as mean ± standard deviation (Mean ± SD) and the data were compared using Student's 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 the Pasteur Institute, Tehran, Iran.

Procedures

Production and quality control of [177]LuCl₃ solution

[177]Lu was produced by irradiation of a natural Lu_2O_3 target (1 mg) at a thermal neutron flux of approximately 4×10^{13} n/cm²·s for 5 d at the Tehran Research Reactor (TRR) according to reported procedures [7] in the Tehran Research Reactor. The irradiated target was dissolved in 200 μl of 1.0 M HCl to prepare [177]LuCl₃ and diluted to an appropriate volume with ultra pure water, to produce a stock solution of a final volume of 5 ml. The mixture was filtered through a 0.22 μm biological filter and sent for use in a radiolabeling step. For radionuclidic purity determination, the samples were checked

by gamma-ray spectroscopy on an HPGe detector for 5 h basing on the two major photons of ^{177}Lu (6.4% of 0.112 MeV and 11% of 0.208 MeV). The radiochemical purity of ^{177}LuCl$_3$ was checked using two solvent systems for ITLC (A: 10 mM diethylenetriaminopetaacetic acid (DTPA) pH.4 and B: ammonium acetate 10% : methanol (1:1)).

Labeling of bleomycin with ^{177}LuCl$_3$

Radiolabeling of bleomycin using cation solution was performed according to previously reported methods [8]. Briefly, ^{177}LuCl$_3$ (74–150 MBq) dissolved in 0.5–2 mL of acidic medium (1 M 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 (3 mg/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 2 h. 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 ^{177}Lu-BLM

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

Stability of ^{177}Lu-BLM complex in the final product

Stability tests were based on previous studies performed for other radiolabeled bleomycins [11]. A sample of ^{177}Lu-BLM (18–180 MBq) was kept at room tempera-ture for 48 h and checked by radio thin-layer chromatography (RTLC) every 4 h. A micropipet sample (5 μL) was taken from the shaking mixture and the ratio of free radio-lutetium to ^{177}Lu-BLM was checked by ITLC in a mixture of 10 mmol/L DTPA solution as mobile phase to discriminate free lutetium from radio-labeled compound.

Serum stability studies

500 μL of freshly prepared human serum was added to 37 MBq (100 μL) of ^{177}Lu-BLM and the resulting mixture was incubated at 37°C for 24 h. Aliquots (5 μL) were analyzed by ITLC up to 24 h of incubation to determine stability of the complex.

Biodistribution of ^{177}LuCl$_3$ and ^{177}Lu-BLM in wild-type rats

^{177}LuCl$_3$ and ^{177}Lu-BLM were administered to separate wild-type rat groups. A volume (50–100 μL) of ^{177}Lu--BLM or ^{177}LuCl$_3$ 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 ^{177}LuCl$_3$ and 2, 24 h and 5 d for ^{177}Lu-BLM), and the ID/g% of different organs was calculated as percentage of injected dose (based on area under the curve of 112 keV peak) per gram.

Results

The radionuclide was prepared in a research reactor according to regular methods with a range of specific activity 2.6–3 GBq/mg for radiolabeling use. The obtained radionuclidic purity was 99.98% (Fig. 2).

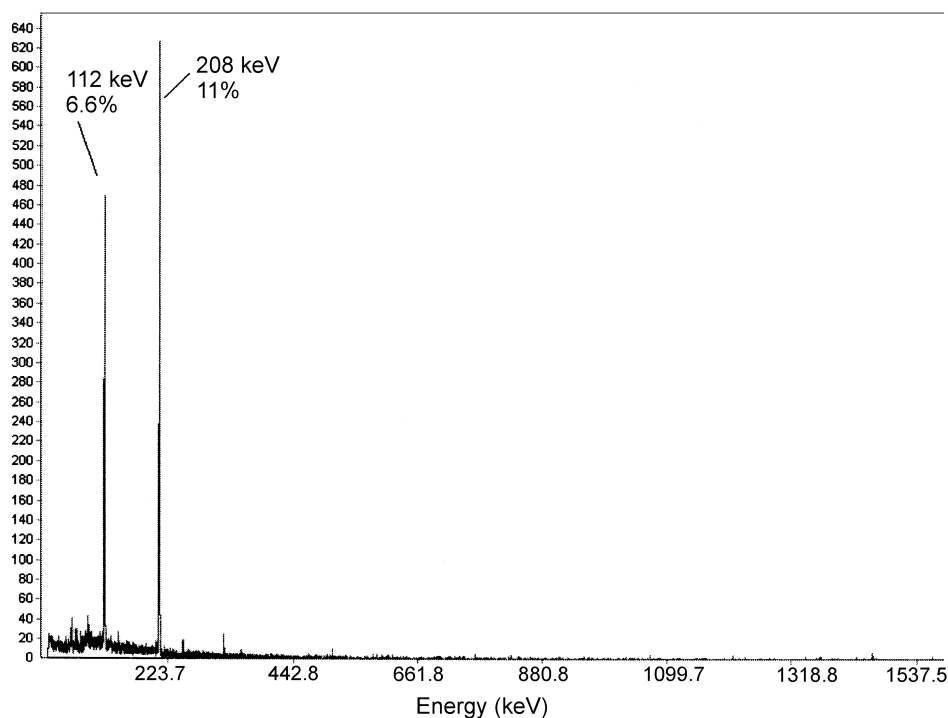The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated

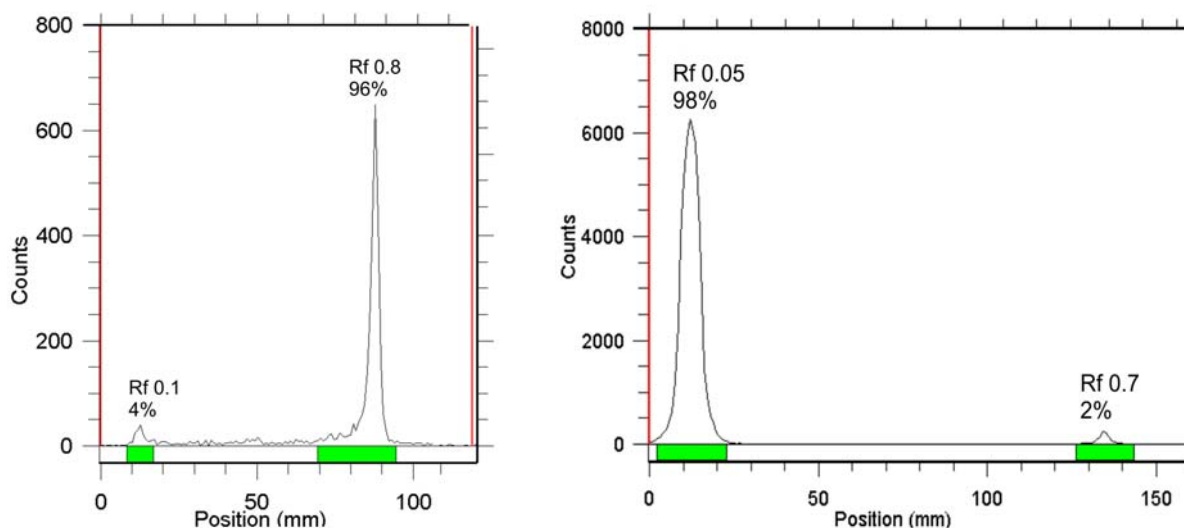**Fig. 2.** Gamma-ray spectrum for Lu-177 chloride solution used in this study.

Fig. 3. ITLC chromatograms of ^{177}LuCl$_3$ solution in DTPA solution (pH 5) (left) and 10% ammonium acetate : methanol (1:1) solution (right) using Whatman no. 2.

for obtaining the desired pH and volume followed by sterile filtering. The radiochemical purity of the ^{177}Lu solution was checked in two solvent systems, in 10 mM DTPA, free Lu^{3+} cation is complexed to more lipophilic LuDTPA form and migrates to higher R_f, while a small radioactive fraction remains at the origin which could be related to other Lu ionic species, not forming LuDTPA complex, such as LuCl$_4^-$ etc. and/or colloids.

On the other hand, a 10% ammonium acetate : methanol mixture was also used for the determination of radiochemical purity. In this solvent system, the fast eluting species were possibly Lu-177 cations, other than Lu^{3+} and the remaining fraction at R_f 0 was a possible mixture of Lu^{3+} and/or colloids. The difference in values of impurity in the two solvent systems is possibly due to the presence of colloidal impurity in the sample (Fig. 3).

Preparation of ^{177}Lu-BLM

In order to obtain the highest specific activity in a 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 LuCl$_3$ for instance), while 25°C was considered a 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 (Figs. 4 and 5).

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 Fig. 1 the pharmaceutical sample is mainly composed of two components with the reported ratio mixture [16], considering the molar ratio, a mean molecular weight of 1495.22 can be calculated, resulting in a specific activity of 740 GBq/mmole in optimized

radiolabeling conditions. The labeling step took about 24 h. In all radiolabeling procedures ($n = 5$), the labeling yield was over 94%. The ratio of ^{177}Lu-BLM peaks at R_fs of 0.1 to free Lu^{3+} radiopeak (R_f: 0.9) was considered as the radiochemical yield using solvent system A (Fig. 6).

For optimization of the labeling conditions at room temperature, the best pH for the labeling step was 5.5–7. In basic conditions the radiochemical yield decreased drastically due to the degradation of bleomycin to less soluble compounds [19].

The final radiolabeled complex diluted with normal saline was then passed through a 0.22 μm (Millipore) filter for sterilization. Incubation of ^{177}Lu-BLM in freshly prepared human serum for 24 h at 37°C showed no loss of ^{177}Lu from the complex at least for 48 h.
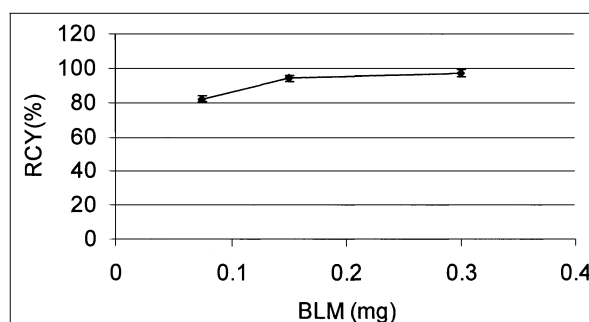

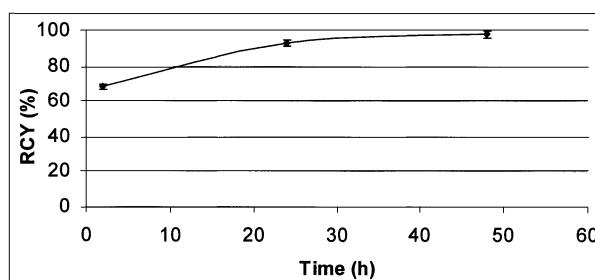**Fig. 4.** Radiochemical yield (RCY) of Lu-BLM in radiolabeling against BLM amounts at 25°C in 24 h.

Fig. 5. Radiochemical yield (RCY) of Lu-BLM (0.15 mg) in radiolabeling against time at 25°C.
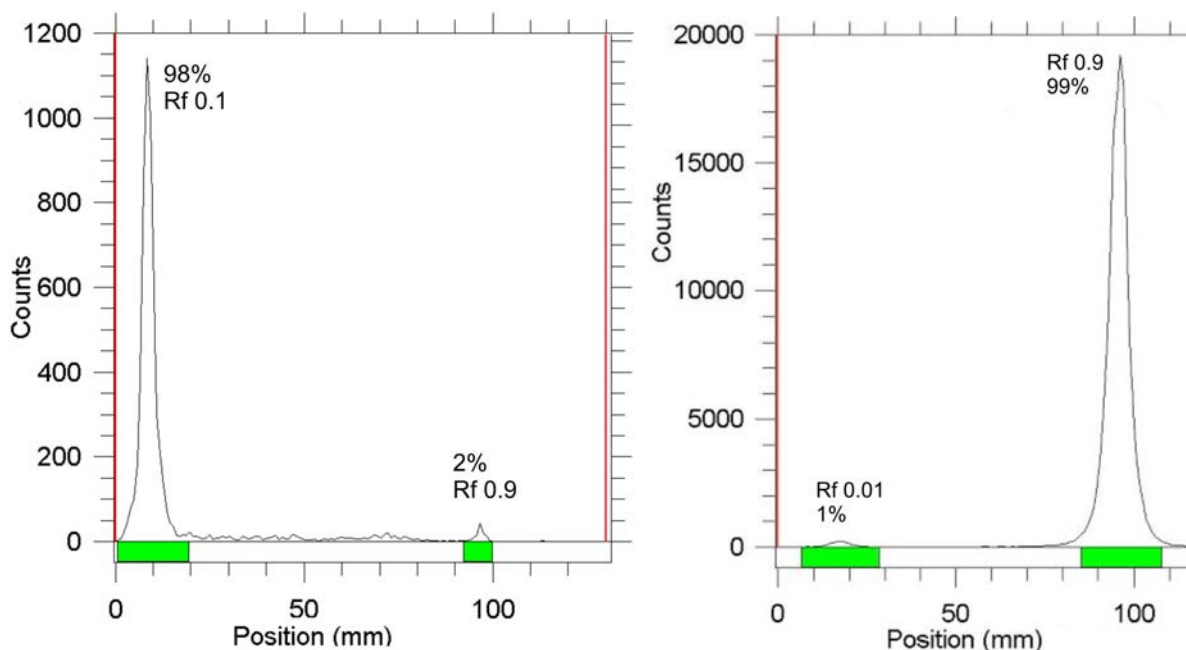
Fig. 6. Radio chromatogram of free Lu^{3+} cation (right) and ^{177}Lu-BLM (left) in 10 mmol/L DTPA solution (pH 5) in optimized conditions.

Biodistribution studies for free ^{177}Lu cation in rats

The animals were sacrificed by CO_2 asphyxiation at selected times after injection (2, 4, 24 and 48 h).

Dissection began by drawing blood from the aorta followed by removing heart, spleen, muscle, bone, kidneys, liver, intestine, stomach, lungs and skin samples. The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g) (Fig. 7). The liver uptake of the cation is comparable to many other radiometals mimicking ferric cation accumulation, about 3% of the activity accumulates in the liver after 48 h. The transfer-in-metal complex uptake and final liver delivery looks the possible route of accumulation.

The blood content is low at all time intervals and this shows the rapid removal of activity in the circulation. Lung, muscle and also skin do not demonstrate significant uptake, and this is in accordance with other cations accumulation. A 4% bone uptake is observed for the cation which remains almost constant after 48 h (data not shown). Spleen also shows a significant uptake, possibly related to a reticuloendothelial uptake. Kidney plays an important role in ^{177}Lu cation excretion, especially after 24 h.

The accumulation of ^{177}Lu-BLM is demonstrated in Fig. 8. The liver and kidney were the major accumulation sites of the radiolabeled bleomycins, which have similar biokinetics to free BLMs. A major route of excretion for the tracer was the urinary tract similar to BLM.
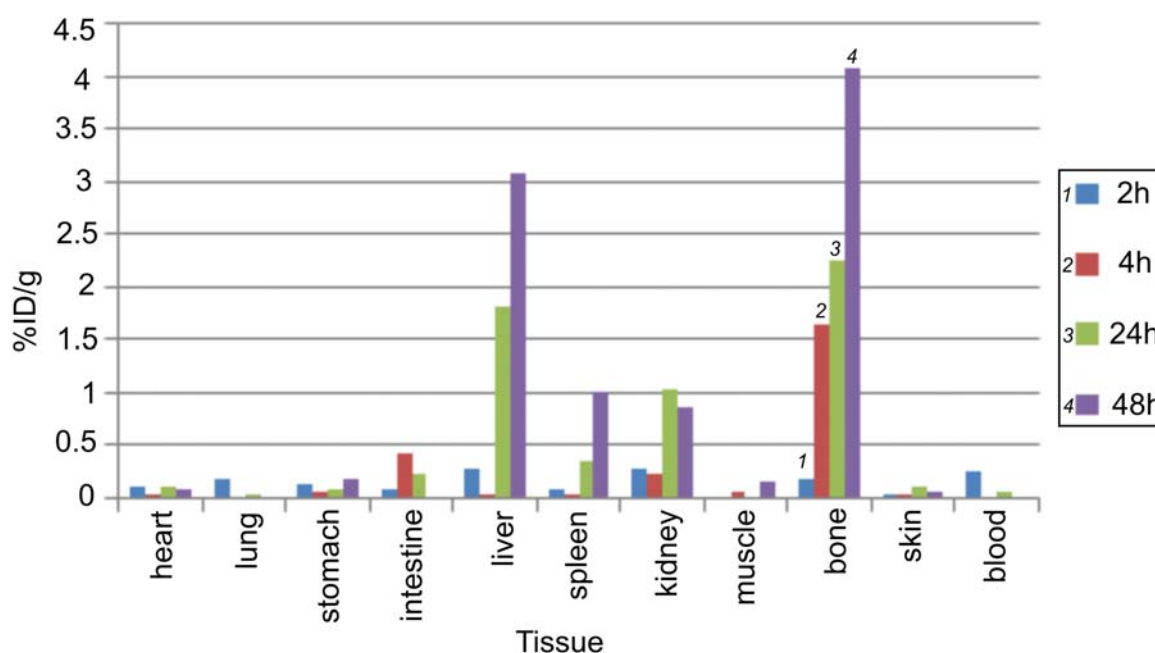

Fig. 7. Percentage of injected dose per gram (ID/g%) of $^{177}LuCl_3$ in wild-type rat tissues at 2, 4, 24 and 48 h post injection.
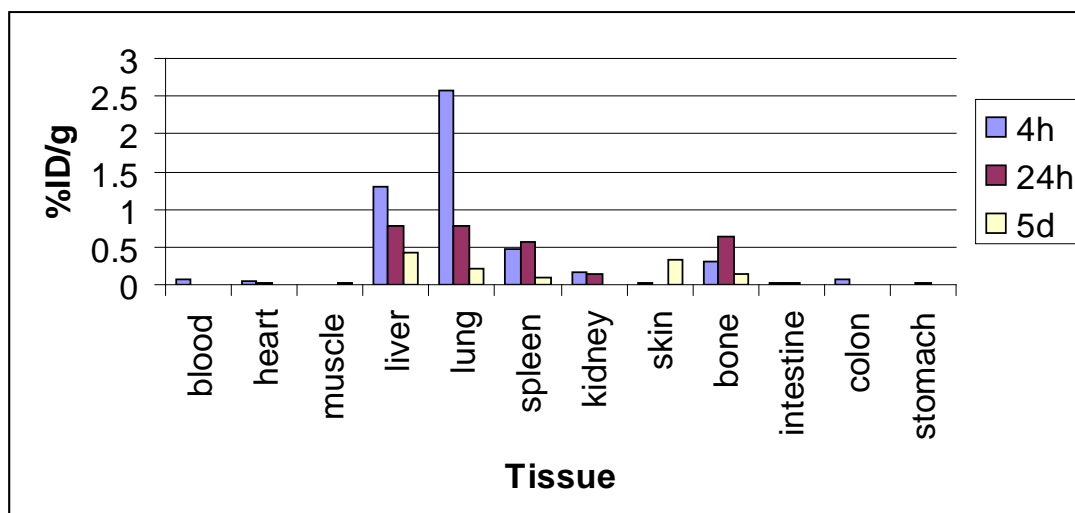
Fig. 8. Percentage of injected dose per gram (ID/g%) of ^{177}Lu-BLM in wild-type rat tissues at 4, 24 h and 5 d post injection.
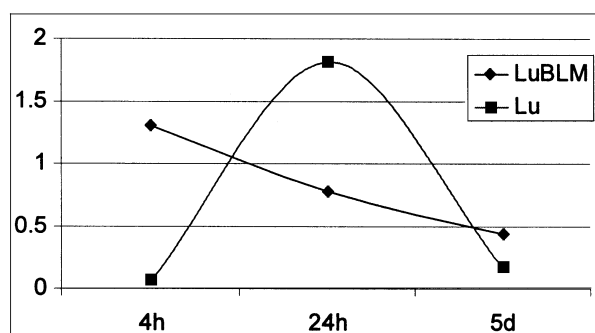

Fig. 9. Comparative liver activity for ^{177}Lu-BLM and [177]LuCl₃ in wild-type rats.

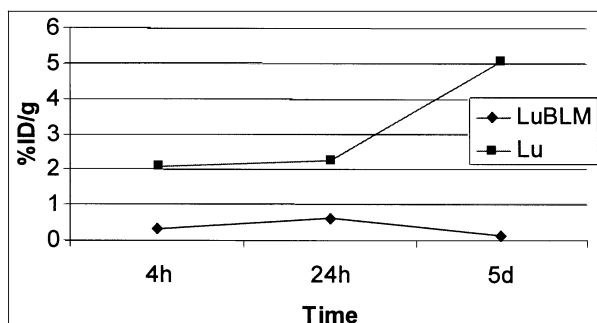**Fig. 10.** Comparative bone activity for ^{177}Lu-BLM and [177]LuCl₃ in wild-type rats.

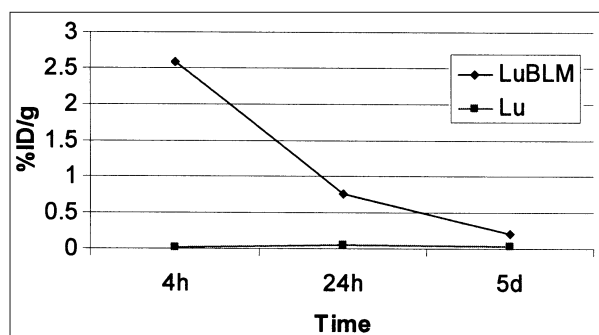**Fig. 11.** Comparative lung activity for ^{177}Lu-BLM and [177]LuCl₃ in wild-type rats.

Discussion

Comparison of vital organs uptake for ^{177}LuCl$_3$ and ^{177}Lu-BLM demonstrates the kinetic pattern difference for both species. ^{177}Lu cation is accumulated in the liver in the first 24 h post injection, and it can be assumed that later the activity is excreted from liver via the biliary tract, while ^{177}Lu-BLM is excreted through kidneys with an exponential rate in 5 d (Fig. 9).

The bone uptake is a result of the presence of the ^{177}Lu cation in circulation. For ^{177}Lu-BLM, bone uptake is almost unchanged during 5 d, which can be a result of small amounts of free Lu in the injected sample or complex dissociation, while ^{177}Lu reaches its maximum after 5 d (Fig. 10).

In the case of lung almost no detectable activity was accumulated as already shown for most of other radiometals, while LuBLM is mainly deposited in the lung due to reported side effects for this antibiotic (Fig. 11).

Summary

In optimized conditions, total labeling and formulation of ^{177}Lu-BLM took about 24 h, with a radiochemical yield higher than 98%. The radiolabeled complex was stable in aqueous solutions for at least 48 h and no significant amount of other radioactive species was detected by ITLC. Trace amounts of ^{177}LuCl$_3$ ($\approx 2\%$) 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 5 d and a significant accumulation took place in the liver, spleen and kidneys which is in accordance with the biodistribution of other reported radiolabeled bleomycin compounds. ^{177}Lu-BLM is a potential therapeutic compound and our experiments on this compound have shown a satisfactory quality, and stability suitable for future therapeutic studies.

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