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ORIGINAL PAPER

# Development of $^{62}$Zn bleomycin as a possible PET tracer

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**Abstract** Bleomycin (BLM), labeled with radioisotopes, is widely used in therapy and diagnosis. In this study, BLM was labeled with $^{62}$Zn for oncologic PET studies. The complex was obtained at pH = 2 in saline at 90°C in 25 min. Radio-TLC showed an overall radiochemical yield of 95–97% (radiochemical purity > 97%). Stability of complex was checked *in vitro* in mice and human plasma/urine. Preliminary *in vivo* studies were performed to determine complex stability and distribution of $^{62}$Zn BLM in normal and fibrosarcoma-bearing mice. $^{62}$Zn BLM accumulated significantly in induced fibrosarcoma tumors in mice according to biodistribution/imaging studies. $^{62}$Zn BLM can be used in PET oncology studies due to its suitable physicochemical properties as a diagnostic complex *in vitro* and *in vivo*. Further studies should be performed for evaluation of the complex behavior in larger mammals.

**Key words** PET • pharmacokinetics • biodistribution • $^{62}$Zn • bleomycin

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## Introduction

Several radiolabeled bleomycin (BLM) derivatives have been developed for imaging and/or therapy of neoplastic tissues. The most important compounds contain $^{111}$In [3], $^{57}$Co [14], $^{99m}$Tc [16] and $^{57}$Fe salts [11]. Technetium complexes of bleomycin did not form any suitable tracers for imaging due to their low radiochemical yield, while trivalent radioisotopes like $^{57}$Co and $^{111}$In afforded stable complexes [14]. Recently some other new complexes, like $^{105}$Rh, have been studied for therapeutic purposes [2]. $^{62}$Zn (HL = 6.9 h, EC: 3%, $\beta^+$: 97%) is a rather long half-life PET radioisotope mostly used in preparation of $^{62}$Zn/$^{62}$Cu generators [4], but its direct use has not been reported in labeling or imaging studies. $^{62}$Zn-labeled bleomycin preparation had been once reported without further biological studies [12]. The aim of this study was to investigate the possibility of labeling bleomycin with zinc-62 for use in positron emission tomography. Due to interesting properties and increasing importance of PET radiotracers, we optimized complex formation conditions with bleomycin, in order to develop $^{62}$Zn BLM as a tumor imaging agent. We report preparation, optimization, stability, bio-stability, formulation and tumor imaging studies of $^{62}$Zn bleomycin complex.

## Experimental

### Materials

Chemicals were purchased from Aldrich Chemical Company, Milwaukee, WI. Bleomycin sulfate (BLM-S)

was a pharmaceutical sample purchased from Nippon Kayaku Laboratories, Japan. Thin-layer chromatography (TLC) was performed on silica gel polymer-backed (F1500/LS 254, 20 × 20 cm, TLC Ready Foil, Schleicher and Schuell). Methanol and normal saline used for labeling were of high purity. A mixture of 10% ammonium acetate and methanol (1:1 v.v) was used as eluent. Radio-chromatography was performed by counting 5 mm-long slices of polymer-backed silica gel paper using a Canberra high purity germanium detector (model GC1020-7500SL). All calculations and TLC counting was performed based on the 511 keV peak. Animal experiments were carried out in compliance with protocols and guidelines.

## Methods

### Preparation of $^{62}ZnCl_2$ from natural copper solid target

$^{62}ZnCl_2$ was prepared by 30 MeV proton bombardment of a natural electroplated copper-target in a 30 MeV cyclotron (Cyclone-30, IBA) based on a method described previously [13]. Briefly, after dissolution of the irradiated target in 8N $HNO_3$, the solution was heated under a flow of nitrogen until a precipitate was formed. The residue was rinsed 2 times by distilled water (10 ml) and a portion of 2N HCl was added and mixed gently. The solution was passed through a cation exchange resin (Dowex 1 × 8) followed by washing the column with HCl 2N solution. Purity of the zinc chloride solution was checked by polarography using a Metrohm 456 system and colorimetric assay (formation of Cu-dithisone complex). This solution was used directly in the labeling step.

### Labeling of bleomycin with $^{62}ZnCl_2$

Zinc-62 chloride ($0.93–1.85 \times 10^7$ Bq) dissolved in acidic media obtained above (0.5–2 ml) was transferred in to a 2 mL-vial and pH was adjusted using HCl and/or NaOH (pH = 1–7) for optimizing the best pH for complexation. The mixture was evaporated by slight warming under a nitrogen flow. A mixture of BLM (0.25–2.5 mg) in normal saline (0.1 mL) was then added. This mixture was heated at different temperatures (25, 50, 80 and 100°C). The mixture was cooled in an ice bath and rapidly used for testing. The radioactive solution was checked for radiochemical purity by polymer-backed silica gel layer using a mixture of 10% ammonium acetate:methanol (1:1 v.v) as the mobile phase. Radio thin-layer chromatography showed two major and distinct radio peaks at the $R_f$s of 0.40 and 0.70. The radiochemical yields (> 95% in each case) was also determined by the RTLC method. These analyses were carried out after labeling. The final solution was then passed through a 0.22 μm filter and pH was adjusted to 5–7 by the addition of sodium acetate (1M) buffer. Gamma spectroscopy of the final sample was obtained by an HPGe detector and showed a radionuclide purity higher than 98%. Bacterial endotoxin test was performed using a commercial LAL kit. No microbial and/or fungal growth were observed in any incubated cultures up to 72 hours after incubation in a suitable incubator, showing acceptable sterility.

### Stability of Zn-62 bleomycin complex in final product

A sample of $^{62}Zn$ BLM ($1.85 \times 10^7$ Bq) was kept at room temperature for 24 h while checked by RTLC at various time intervals (2, 4, 8, 12 and 24 h). A micropipet sample (50 μL) was taken from the shaken mixture and the ratio of free radiozinc to $^{62}Zn$ BLM was checked by radio thin-layer chromatography (eluent: 10%$NH_4OAc$:methanol, 1:1 v.v).

### Stability of Zn-62 bleomycin complex in human and mice serum *in vitro*

A mixture of 5 parts of serum and one part of the radiopharmaceutical ($0.74 \times 10^7$ Bq) was shaken in a 37-degree incubator under nitrogen atmosphere. A micropipet sample (50 μL) was taken from the shaken mixture every 30 min. The ratio of free radiozinc ($R_f$ = 0) to $^{62}Zn$ BLM ($R_f$ = 0.4 and 0.7) was checked by radio thin-layer chromatography (eluent: $NH_4OAc$:methanol, 1:1, v.v).

### Stability of Zn-62 bleomycin complex in human urine

A mixture of 5 parts of healthy human urine and one part of the radiopharmaceutical ($0.74 \times 10^7$ Bq) was incubated at 37°C under nitrogen atmosphere. A micropipet sample (50 μL) was taken from the shaken mixture every 30 min. The ratio of free radiozinc ($R_f$ = 0) to $^{62}Zn$ BLM ($R_f$ = 0.4 and 0.7) was checked by TLC.

### Cell cultures

Cell lines of murine fibroblastoma were used for experiments. For each culture $1–2 \times 10^4$ cells were seeded into a 75 $cm^3$ flask containing 20 ml of medium supplemented with 10% fetal bovine serum and 1% glutamine. Cells were incubated at 37°C in 5% $CO_2$. The cell line was maintained in exponential growth phase and passaged twice per week.

### Animal studies

Fibrosarcoma cells (about $10^4$) were injected SC to the dorsal area of Balb/C mice weighing 20–25 g. After 14 days, the tumor weighed 0.7 g and was not grossly necrotic. The distribution of $^{62}ZnCl_2$ and $^{62}Zn$ BLM among tissues were determined for untreated mice and for mice with fibrosarcoma. A volume (0.1 ml) of final $^{62}Zn$ BLM solution containing ($7.4–14.8 \times 10^5$ Bq) radioactivity (≤ 6 μg of bleomycin in 50 μL) was injected into the dorsal tail vein. The total amount of radioactivity injected into each mouse was measured by

**Table 1.** Biodistribution of $^{62}$Zn BLM in organs of tumor-bearing mice ($n$=5) (%ID/g tissue); Avg.: average, SD: standard deviation

| Organ | Time [h] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 4 | | 8 | |
| | Avg. | SD | Avg. | SD | Avg. | SD | Avg. | SD |
| Blood | 2.49 | 0.44 | 3.92 | 0.54 | 4.46 | 0.26 | 4.99 | 0.27 |
| Liver | 3.1 | 0.23 | 2.99 | 0.6 | 3.44 | 0.71 | 3.96 | 0.78 |
| Kidney | 1.0 | 0.06 | 3.66 | 0.88 | 7.30 | 1.12 | 7.86 | 1.15 |
| Stomach | 1.3 | 0.08 | 2.7 | 0.54 | 4.70 | 0.84 | 5.12 | 1.01 |
| Colon | 0.44 | 0.05 | 0.85 | 0.05 | 1.21 | 0.05 | 1.34 | 0.09 |
| Stool | 1.62 | 0.07 | 1.92 | 0.1 | 2.18 | 0.13 | 3.52 | 0.19 |
| Bladder | 1.12 | 0.06 | 2.39 | 0.15 | 3.48 | 0.21 | 4.84 | 0.35 |
| Sternum | 0.81 | 0.02 | 0.78 | 0.02 | 0.73 | 0.05 | 0.67 | 0.02 |
| Lung | 1.1 | 0.03 | 1.74 | 0.55 | 2.20 | 1.94 | 2.64 | 0.54 |
| Skin | 1.65 | 0.09 | 1.75 | 0.12 | 1.76 | 0.15 | 1.81 | 0.12 |
| Muscle | 7.26 | 1.11 | 5.12 | 0.91 | 3.32 | 0.42 | 1.87 | 0.25 |
| Tumor | 4.33 | 0.85 | 5.32 | 0.99 | 5.34 | 0.92 | 5.77 | 1.01 |

counting the 1-ml syringe before and after injection in a radiometer with fixed geometry. The animals were sacrificed by ether asyxphycation at selected times after injection, the tissues were weighed and their specific activities were determined with a γ-ray scintillation as percentage of injected dose per gram of tissues (Tables 1 and 2).

### Imaging of $^{62}$Zn BLM in tumor bearing mice

Fibrosarcoma-bearing mice were used for tumor imaging when the tumors had reached a size of 1.5–2 cm at 2–3 weeks after its induction. Images were taken 1, 2, 4, 6 and 8 h after administration of the radiopharmaceutical in the coincidence mode by a Dual-Head SPECT system (SMV, France, Sopha DST-XL). The mouse-to-high energy septa distance was 12 cm. Images were taken from both the normal and tumor bearing mice. The useful field of view (UFOV) was 540 mm × 400 mm. The spatial resolution in the coincidence mode was 10 mm FWHM at the CFOV. Sixty four projections were acquired for 30 s per view with a 64 × 64 matrix.

## Results and discussion

Bleomycin is an antineoplastic agent widely used in therapy [6]. This compound produces suitable and stable complexes with cations like $Mg^{2+}$, $Ca^{2+}$, $Fe^{2+}$,

**Table 2.** Biodistribution of $^{62}ZnCl_2$ in organs of tumor-bearing mice ($n$=5) (%ID/g tissue); Avg.: average, SD: standard deviation

| Organ | Time [h] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 4 | | 8 | |
| | Avg. | SD | Avg. | SD | Avg. | SD | Avg. | SD |
| Blood | 1.33 | 0.04 | 1.02 | 0.14 | 0.35 | 0.06 | 0.10 | 0.07 |
| Liver | 0.12 | 0.03 | 0.19 | 0.06 | 0.34 | 0.02 | 0.56 | 0.18 |
| Kidney | 1.0 | 0.06 | 1.16 | 0.18 | 1.30 | 0.12 | 0.86 | 0.15 |
| Stomach | 0.63 | 0.08 | 2.70 | 0.54 | 1.70 | 0.44 | 1.12 | 0.01 |
| Colon | 0.64 | 0.05 | 1.25 | 0.05 | 0.84 | 0.15 | 0.34 | 0.09 |
| Stool | 0.62 | 0.07 | 1.92 | 0.10 | 0.58 | 0.13 | 0.52 | 0.09 |
| Bladder | 3.12 | 0.56 | 2.79 | 0.15 | 2.48 | 0.21 | 1.84 | 0.15 |
| Sternum | 0.80 | 0.02 | 0.73 | 0.02 | 0.79 | 0.05 | 0.72 | 0.02 |
| Lung | 1.02 | 0.03 | 0.94 | 0.25 | 0.80 | 0.04 | 0.64 | 0.14 |
| Skin | 0.63 | 0.09 | 0.75 | 0.12 | 0.75 | 0.15 | 0.81 | 0.16 |
| Muscle | 0.26 | 0.11 | 0.32 | 0.11 | 0.29 | 0.02 | 0.17 | 0.05 |

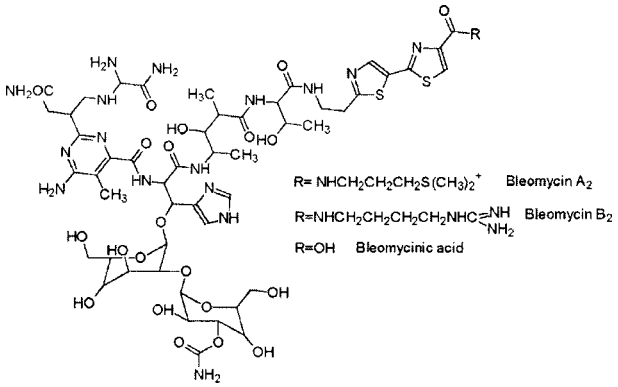


**Fig. 1.** Structures of commercial bleomycin components, BLMA2, ionic component, BLMB2 a rather polar component and bleomycinic acid, the hydrolysed form.

$In^{3+}$ (Fig. 1) [17]. It is believed that these antibiotics interfere with DNA as false nucleotides assuming the dithiazole moiety acts like a purine base [5]. On the other hand, these compounds are activated by a cation insertion as an antineoplastic agent. The whole complex then can act like a peroxidase system by the production of hydrogen peroxide, resulting in DNA decomposition [15]. Thus, labeling of bleomycins with bi/trivalent radioisotopes produces pharmacologically active compounds carrying a diagnostics and/or therapeutic radioisotope [9]. $^{111}$In-bleomycin has been widely used as a therapy/diagnostic agent since the 1970s up to now [7, 8]. Zinc cation coordinates with at least five nitrogen atoms of bleomycin, based on NMR studies [18, 19]. This coordination forms a rather stable complex. Cell toxicity of Zn-bleomycin has been studied and tested in human and different animals [13]. The antitumor activity of Zn-bleomycin complex has been elucidated in some human tumor models [10] suggesting the possibility of application of radiozinc-bleomycin complexes in human tumor imaging.

## Labeling

Because of several polar functional groups in its structure, labeling of bleomycin with a cation does not affect its chromatographic properties, so that the labeled and unlabeled bleomycin almost migrate to the same $R_f$. The more polar bleomycin fraction, i.e., bleomycin $A_2$ correlates with the lower $R_f$, the two other polar fractions come at the close $R_f$s (bleomycin $B_2$ and bleomycinic acid). In all radiolabeling procedures ($n$ = 5), the area under curve ratio of the two peaks was constant, showing the isomeric ratio of the two bleomycin chromatogram peaks (Fig. 2). In order to obtain the best labeling reaction conditions, the complex formation was optimized for pH, temperature, time, and the amount of bleomycin.

In order to start the optimization, a random temperature (80°C) was chosen. At this temperature, the best pH for the labeling step was 2, while at higher pHs (5–6) the radiochemical yield is increasing again due to the formation of different labeled species (Fig. 3). In basic conditions, the radiochemical yield decreased

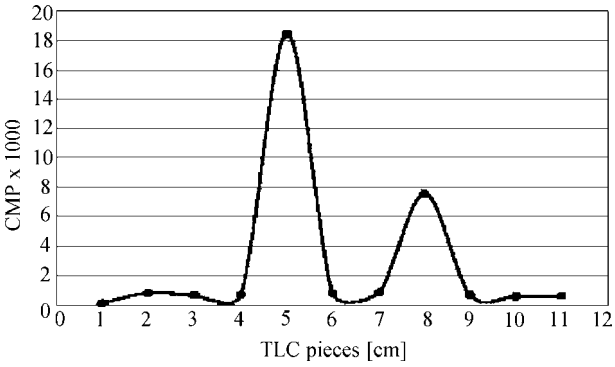


**Fig. 2.** Radio-chromatogram of a $^{62}$Zn BLM sample in optimized condition.

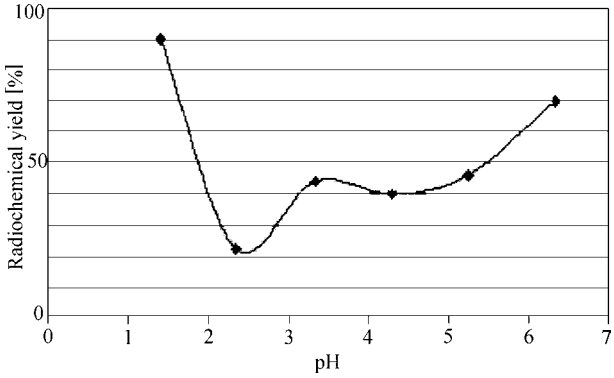


**Fig. 3.** Effect of pH on radiochemical yield of $^{62}$Zn BLM at 80°C.

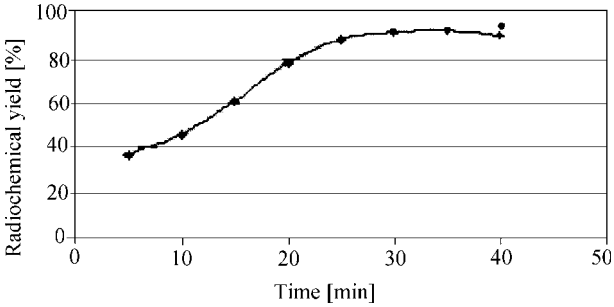


**Fig. 4.** Effect of time on radiochemical yield of $^{62}$Zn BLM at 80°C.

drastically due to the degradation of bleomycin to less soluble compounds.

At the optimum reaction temperature and pH, the yield reached a maximum within 25 min (Fig. 4), and stayed constant for longer reaction times up to 1 hour. Increasing the ratio of bleomycin to radioactivity increased the labeling yield, presumably due to more available chelate in solution (Fig. 5).

For optimizing the temperature, the reaction mixture was heated up to 90–100°C, while the yield increased. Further heating reduced the radiochemical yield due to decomposition of bleomycin and/or product (Fig. 6).

Twenty five to forty percent of the activity remained on 0.22 mm millipore filters when filtration was used to sterilize the product. The thermal stability of $^{62}$Zn BLM was excellent so that autoclaving a $^{62}$Zn bleomycin preparation showed no change in the amount of free

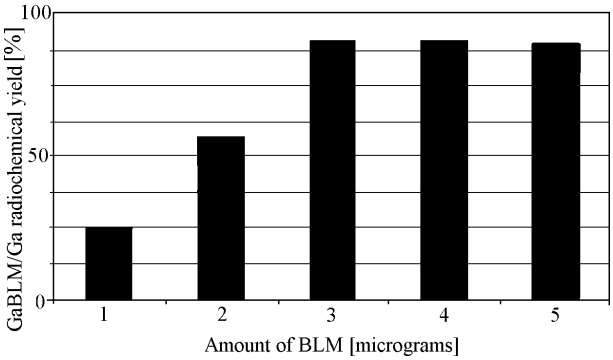


**Fig. 5.** Effect of amount of BLM on radiochemical yield of $^{62}$Zn BLM at 80°C.

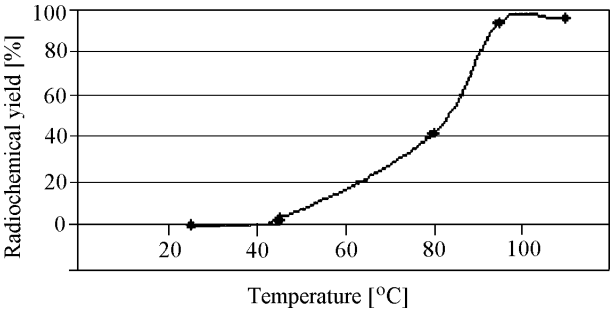


**Fig. 6.** Effect of temperature on radiochemical yield of $^{62}$Zn BLM in optimized conditions.

zinc present. The biological stability of $^{62}$Zn BLM was high enough to perform scanning. The patterns for $^{62}ZnCl_2$ and $^{62}$Zn BLM were not changed in 24 h.

Presence of 3–5% free zinc on the RTLC before and after autoclaving indicates that the preparation may be sterilized by this technique. Since zinc-62 decays to copper-62, another PET complex, $^{62}$Cu BLM, is produced which retains tumor affinity. The biological stability of $^{62}$Zn BLM was high enough to perform scanning.

### Biodistribution in animal tissues

Liver and spleen uptake increased 2–4 h after administration of $^{62}$Zn BLM. Lung uptake increased after 4 h. After 2 h, the radioactivity of bladder and kidney increased and maintained constant for the next few hours, like that of unlabeled bleomycin reported in pharmaceutical texts [1], suggesting the stable incorporation of $^{62}$Zn into bleomycin core. These observations were quite different from the biodistribution of $^{62}ZnCl_2$ which shows rapid washout from kidneys in the first 2–4 h. A late increase in liver uptake was observed that can be due to the accumulation of metalloproteins in this tissue (Tables 1 and 2). Our results were similar in some aspects with *in vivo* biodistribution experiments previously done for $^{111}$In-bleomycin. $^{62}$Zn bleomycin is rapidly tagged in tumor and scanning can be done in rather short times after I.V. injection. Lower half-life of $^{62}$Zn in contrast to $^{111}$In is another important advantage leading to lower radiation exposure to patients.

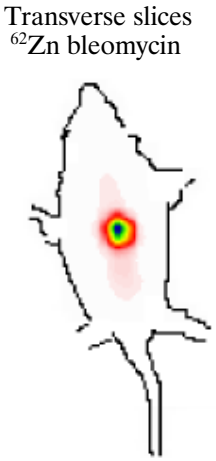


**Fig. 7.** Co-incidence images of fibrosarcoma-bearing mice 2 h after I.V. injection of $^{62}$Zn BLM.

### Imaging

$^{62}$Zn BLM imaging performed in the tumor-bearing mice showed a distinct accumulation of the radiotracer in the tumor, while in the first and second hour a high background in liver and kidney was observed (Fig. 7).

## Conclusion

Total labeling and formulation of $^{62}$Zn BLM took about 60 min, with a yield of 95–97%. A suitable specific activity product was formed via insertion of radiozinc cation. No unlabelled and/or labeled by-products were observed upon TLC analysis of the final preparations. Trace amounts of $^{62}ZnCl_2$ (< 3%) were detected by paper chromatography. HPLC and TLC showed that radiochemical purity of the $^{62}$Zn-labeled components was > 95%. Since the physical half-life of $^{62}$Zn is longer than most of other PET tracers, $^{62}$Zn bleomycin, is a PET tracer with a suitable half-life, benefiting from PET advantages. Finally, the high chemical stability of the radiopharmaceutical form makes it a suitable possible PET tracer for use in the neighborhood PET centers.

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