

Radiolabeling and evaluation of two ^{177}Lu -labeled *bis*-phosphonates

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ABSTRACT

Introduction: Bone pain palliation therapy is a mandate in handling end-stage cancer patients. The development of new ligands with higher stability, better pharmacokinetics and lower unwanted tissue uptakes (liver and GI) is still ongoing.

Methods: In this work Lu-177 labeled (3-amino-1-hydroxypropane-1,1-di-yl)-*bis*-(phosphonate) (^{177}Lu -pamidronate; ^{177}Lu -PAM), and (3-amino-1-hydroxybutane-1,1-di-yl)-*bis*-(phosphonate) (^{177}Lu -alendronate; ^{177}Lu -ALN) complexes were prepared successfully using related ligands and $^{177}\text{LuCl}_3$ at 25°C & 60°C at various ligand:metal ratios for 60-360 min. Lu-177 chloride was obtained by thermal neutron irradiation ($4 \times 10^{13} \text{ n.cm}^{-2}\text{s}^{-1}$) of natural Lu_2O_3 samples. Radiochemical purities of ^{177}Lu - complexes were checked by ITLC and HPLC. Stability studies of final preparation in the presence of human serum were evaluated along with protein binding studies as well as hydroxyapatite (HA) binding test. The biodistribution of ^{177}Lu -complexes and $^{177}\text{LuCl}_3$ in mice were determined for 7 d.

Results: The complexes were obtained in high radiochemical purity ITLC (>97%) and HPLC (>99.9%). Satisfactory stability in presence of human serum and final formulations were obtained ($\approx 90\%$ in 48 h). HA binding assay demonstrated >98% binding from 5-20 mg. The complex protein binding was about 50-58%.

Conclusion: Biodistribution of both complexes demonstrated low bone uptake ratios at all time intervals, for far inferior to ^{177}Lu -EDTMP.

Key words: Lu-177; Pamidronate; Alendronate; Palliation therapy; Biodistribution; Pharmacokinetics

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INTRODUCTION

The progression of various tumors such as prostate (80%), breast and lung carcinoma (50%) usually lead to bone metastases [1] and nearly half of them experience bone pain [2]. In these patients who have progressive disease despite treatment, a systemic bone avid radiopharmaceutical for treatment of widespread bone metastases has potential benefits [3].

Various radiolabeled bisphosphonates have been used in the management of metastatic bone pain including ^{153}Sm -EDTMP, $^{188/186}\text{Re}$ -HEDP, ^{177}Lu -EDTMP and also ^{166}Ho -EDTMP. Although some of these agents have been approved by well-known pharmaceutical legal bodies, however, the final application of the agent around the world is a function of radionuclide availability, physical half life and logistics in drug production. Thus the research and development of bone pain palliation compounds is an on-going research area around the world.

Owing to Lu-177 suitable decay characteristics [$t_{1/2} = 6.73$ d, $E_{\beta\text{max}} = 497$ 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 seems a suitable radionuclide in the development of a Lu-bis phosphonate complexes as possible therapeutic radiopharmaceuticals. Recently many Lu-177 labeled bone avid radiopharmaceuticals have been developed in our country [4], among these agents; Lu-177 EDTMP has been prepared in radiopharmaceutical grade for clinical trials [5] and successfully applied in clinic [6].

The search for the development of new ligands with higher stability, better pharmacokinetics and lower unwanted tissue uptakes (liver and GI) is still ongoing.

Multidentate polyaminopolyphosphonic acid ligands are known to form stable chelates with many metals including lanthanides. (3-amino-1-hydroxypropane-1,1-di-yl)-bis-(phosphonate) (pamidronate; PAM) and (3-amino-1-hydroxybutane-1,1-di-yl)-bis-(phosphonate) (alendronate; ALN) can be suggested as possible carrier moieties for the development of beta emitter-based radiopharmaceuticals for bone pain palliation (Figure 1).

Considering the inhibitory binding affinity constant (Ki) of bisphosphonates used in clinics including etidronate (Ki: 91 μM), ibandronate (116 μM), pamidronate (83 μM), risedronate (85 μM) and zoledronate (81 μM), and the idea of developing bone avid agents based on these ligands is of great interest, however in one study with ^{177}Lu -zoledronate, the resulting complex was not stable enough *in vivo* compared to other therapeutic bisphosphonates due to the existence of an imidazole ring [7]. Thus other

interesting ligands based on affinity constants are considered namely pamidronic acid and alendronic acid containing an amino group which possibly could increase the complex stability.

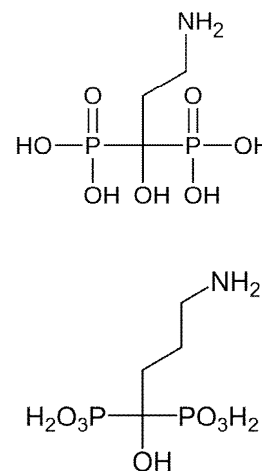


Fig 1. Chemical structures for pamidronic acid (above) and alendronic acid (below).

These compounds are clinically used to prevent osteoporosis, Paget's disease, bone loss due to steroid use, in certain cancers including multiple myeloma. Due to their ability to sequester calcium in bone, they are also used to treat hypercalcemia. Biological half lives for PMD (28.7 h) as well as ALN (26 h) are reported.

In continuation of our research project on the development of ^{177}Lu -labeled bisphosphonates for bone pain palliation therapy [8, 9], in this work, Lu-177 labeled (3-amino-1-hydroxypropane-1,1-di-yl)-bis-(phosphonate) (^{177}Lu -pamidronate; ^{177}Lu -PAM) and (3-amino-1-hydroxybutane-1,1-di-yl)-bis-(phosphonate) (^{177}Lu -alendronate; ^{177}Lu -ALN) complexes was prepared followed by preclinical evaluation including *in vitro/vivo* stability and *post-mortem* biodistribution studies.

METHODS

^{177}Lu was produced with a specific activity of approximately 70-80 mCi.mg^{-1} and radionuclidic purity of 99.98 % by irradiation of natural Lu_2O_3 targeted at a thermal neutron flux of approximately $4 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$ for 5 days at the Tehran Research Reactor (TRR). Whatman No. 3 paper was obtained from Whatman (UK). Sodium alendronate and sodium zoledronate provided by two Iranian Pharmaceutical Companies. Radio-chromatography was performed by Whatman paper using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Analytical HPLC to determine the specific

activity was performed by a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250×4.6 mm (Whatman Co. NJ, USA). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL, Canberra Industries, Inc. CT, U.S.A.) multichannel analyzer and a dose calibrator ISOMED 1010 (Elimpex-Medizintechnik, Austria) were used for counting distributed activity in mice organs. All other chemical reagents were purchased from Merck (Germany). Calculations were based on the 112 keV peak for ^{177}Lu . All values were expressed as mean \pm standard deviation and the data were compared using Student's T-test. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations. 1987. The approval of NSTRI Ethical Committee was obtained for conducting this research. The wild-type mice were purchased from Pasteur Institute of Iran, Karaj; all weighing 20-25 g and were acclimatized at proper rodent diet and 12h/12h day/night light/darkness.

Production and quality control of $^{177}\text{LuCl}_3$ solution

Lutetium-177 was produced by neutron irradiation of 1 mg of natural Lu_2O_3 (99.999% from Aldrich Co., UK) according to reported procedures [10] at Tehran Research Reactor (TRR), General Atomics, USA. The irradiated target was dissolved in 200 μL of 1.0 molL^{-1} HCl, to prepare $^{177}\text{LuCl}_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution of final volume of 5 mL (0.04 molL^{-1}). The mixture was filtered through a 0.22 μm filter for sterilization, Waters, U.S.A. The radionuclidic purity of the solution was tested for the presence of other radionuclides using purity germanium (HPGe) spectroscopy for the detection of various interfering gamma emitting radionuclides. The radiochemical purity of the $^{177}\text{LuCl}_3$ was checked using 2-solvent systems for instant thin layer chromatography (ITLC) [A: 10 mmolL^{-1} diethylene triamine pentaacetic acid (DTPA) pH.5 and B: 10% ammonium acetate:methanol (1:1)].

Radiolabeling of bisphosphonates with $^{177}\text{LuCl}_3$

A stock solution of mono sodium bis phosphonate salts were prepared by dissolution double distilled ultra pure water, to produce a solution of 50 mg.mL^{-1} . For labeling, an appropriate amount of the $^{177}\text{LuCl}_3$ solution containing the required activity (0.1 mL, 5 mCi) was added to the desired amount of Na bis phosphonate solutions (0.3 mL, 1:5; 1:10; 1:15; 1:20; 1:40 and 1:50 ratios for Lu: bis phosphonate). The complex solutions were kept at room temperature for

60-360 min. Also another set of experiment was performed at 60°C warm bath for 60-360 min. The radiochemical purity was determined using ITLC and HPLC. The final solution was passed through a 0.22- μm membrane filter and pH was adjusted to 7-8.5 with 0.05 molL^{-1} phosphate buffer (pH 5.5).

Radiochemical purity of ^{177}Lu -bisphosphonates

Instant thin layer chromatography: A 5 μL sample of the final fraction was spotted on a chromatography Whatman No. 3, paper, and developed in NH_4OH (56%): MeOH (%100): H_2O (%100) (0.2:2:4; v/v/v) as mobile phase mixture.

High performance liquid chromatography: HPLC was performed with a flow rate of 1 mL/min, pressure: 130 kgF/cm^2 for 20 min. HPLC was performed on the final preparation using a mixture of water:acetonitrile 3:2(v/v) as the eluent by means of reversed phase column Whatman Partisphere C_{18} 4.6 \times 250 mm.

Sterility and apyrogenicity of the radiopharmaceutical

Sterility was controlled on a random sampling following decay of radioactivity. The Limulus amoebocyte lysate (LAL) test was used for validation of radiopharmaceutical production according to the European protocol [11].

Stability of ^{177}Lu -bisphosphonates in final formulation

Stability of ^{177}Lu - bisphosphonates in final preparation was determined by storing the final solution at 25°C for up to 48 h and performing frequent ITLC analysis to determine radiochemical purity using Whatman 3 MM chromatography paper or ITLC-SG eluted with NH_4OH (56%): MeOH (%100): H_2O (%100) (0.2:2:4; v/v/v).

In vitro protein binding of ^{177}Lu -bisphosphonates in presence of human serum

In vitro protein binding of ^{177}Lu -bisphosphonates was carried out in human blood by protein precipitation according to published procedure [12]. To 3 mL fresh human plasma, 1mL of the labeled complex was mixed and incubated for 1h at 37°C. Contents of the tube were centrifuged at 3000 rpm for 10 min for separation of serum and blood cells. After mixing approximately equal volume of 10% trichloroacetic acid (TCA), the mixture was centrifuged at 3000 rpm for 10 min. Residue was separated from supernatant and both layers were counted for radioactivity in a well type gamma counter. Protein binding of the complex was expressed as the fraction of

radioactivity bound to protein, in percentage of the total radioactivity.

In vitro stability of ^{177}Lu -bisphosphonates in presence of human serum

Final solution (200 μCi , 50 μL) was incubated in the presence of freshly prepared human serum (300 μL) (Purchased from Iranian Blood Transfusion Organization, Tehran) and kept at 37°C for 2 days. Every 30 min to a portion of the mixture (50 μL), 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 performing frequent ITLC analysis of supernatant using above mentioned ITLC system.

Hydroxyapatite binding assay

The hydroxyapatite binding assay was performed according to the procedure described previously [13] with only a slight modification. In brief, to vials containing 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 mg of solid hydroxyapatite, 2ml of saline solution of pH 7.4 were added and the mixtures were shaken for 1h. Then, 50 ml of the radioactive preparation was added and the mixtures were shaken for 24 h at room temperature. The suspensions were centrifuged, and two aliquots of the supernatant liquid were taken from each vial and the radioactivity was measured with a well-type counter. Test experiments were performed using a similar procedure, but in the absence of hydroxyapatite. The percentage binding of ^{177}Lu to hydroxyapatite (HA) was calculated according to $\text{HB} = 1 - \text{A}/\text{B} \times 100$, where A is the mean radioactivity value of the supernatant sample under study and B is the mean total value of whole activity used.

Biodistribution studies

The biodistributions of free $^{177}\text{Lu}^{3+}$ cation as well as of ^{177}Lu -bisphosphonates were determined in wild type mice. For each compound, 100 μL (150 μCi) of radioactive solution was injected directly to normal mice through caudal vein. The animals (n=3) were sacrificed at selected times after injection (2 h to 7d) and percentage of injected dose in the tissues was determined with a γ -ray scintillation or a dose calibrator.

RESULTS AND DISCUSSION

Radionuclide production

The radionuclide was prepared at a range of specific activity of 3 to 5 $\text{MBq}\cdot\text{mg}^{-1}$ for radiolabeling use,

after counting the samples on an HPGe detector for 5 h, two major photons (6.4% of 0.112 MeV and 11% of 0.208 MeV) were observed.

The radiochemical purity of the ^{177}Lu solution was checked in two solvents. In 10 $\text{mmol}\cdot\text{L}^{-1}$ DTPA aq. solution (solvent 1), free Lu^{3+} is coordinated to more lipophilic moiety as $\text{Lu}(\text{DTPA})^{2-}$ and migrates to higher R_f . Small radioactive fraction remains at the origin could be associated to colloids, since in presence of very strong complexing agent (i.e. DTPA), existence of other ionic species than $\text{Lu}(\text{DTPA})^{2-}$ is impossible. On the other hand, 10% ammonium acetate:methanol mixture (1:1) (solvent 2) was also used for the determination of radiochemical purity. The fast eluting species was possibly Lu^{3+} and other ionic forms of Lu-177 such as LuCl_4^- remained at the origin ($R_f=0$) as well as colloids (Figure 2).

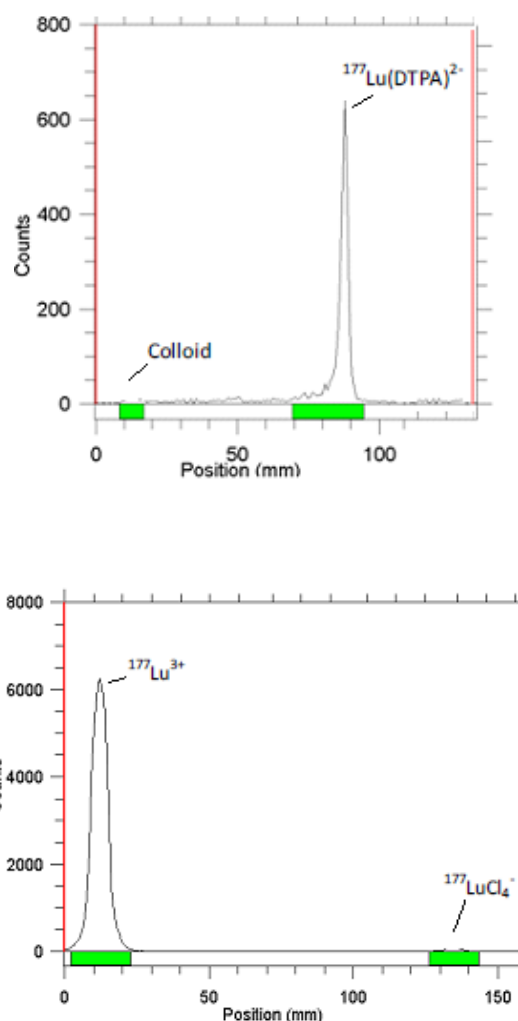


Fig 2. ITLC chromatograms of $^{177}\text{LuCl}_3$ solution in a DTPA solution (pH. 5) (above), 10% ammonium acetate:methanol (1:1; v/v) solution (below).

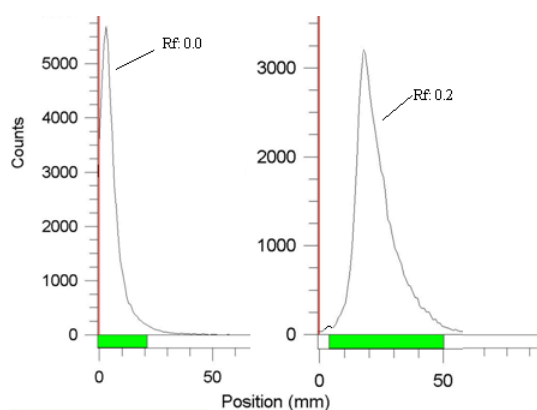


Fig 3. ITLC chromatograms of $^{177}\text{LuCl}_3$ (left) and ^{177}Lu -alendronate solution (right) using $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (0.2:2:4).

The differences in the impurity peaks in the two chromatograms could be in part related to the presence of a colloidal impurity which was insignificant. Also insignificant (about <1%) amount of activity can be attributed to other ionic impurities.

Labeling optimization studies

In order to obtain maximum complexation yields, several experiments were carried out varying different reaction parameters such as ligand concentration, pH, reaction time and temperature.

Ligand concentration was varied between a wide ranges starting from 10 to 50mg/ml for bisphosphonates. It was observed that at room temperature 99% complex formation was achieved with 15 mg/ml of bisphosphonates. Higher ligand amount did not increase the radiochemical purity. The use the 15 mg was considered adequate for the labeling. The best ITLC mobile phase was considered by Whatman No.2 paper using $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (0.2:2:4) as shown in Figure 3.

Although the ITLC studies confirmed the production of the radiolabeled compound, HPLC studies demonstrated the existence of at least two radiolabeled species using scintillation detectors.

A more fast-eluting compound at 0.06 min (scintillation detector) related to 0.08 min peak (UV detector 425 nm, not shown) demonstrated a hydrophilic species compared to lutetium cation. In case of pamidronate two radiolabeled species were eluted at 2.99 and 3.87 min from the column which contained more than 99% of the area under curve (Figure 4).

Also in case of alendronate two radiolabeled species were eluted at 3.02 and 3.88 min from the column which contained more than 99% of the area under curve (Figures 5). In all reaction mixture s the two peaks were present and no effect of ligand

concentration on the peak ratios was observed. It seems that the two complexes possessing close chemical properties are formed in the same stoichiometric ratios, they could be spatial isomers (like; syn, anti,...), however there is no proof to demonstrate that. Since the complexes did not show any significant bone uptake, further efforts to discriminate and determine the two complex structures were not justified.

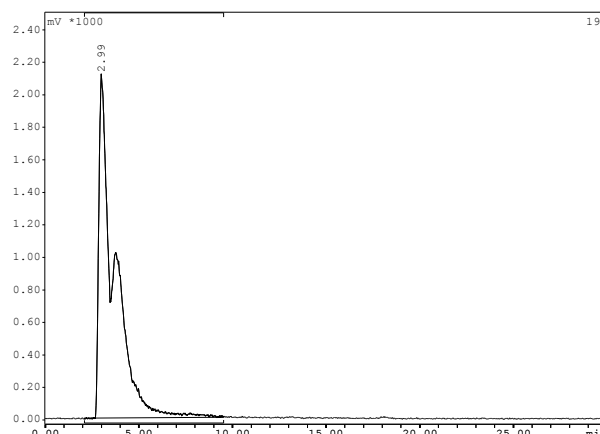


Fig 4. HPLC chromatograms of ^{177}Lu -PMD on a reversed phase column using acetonitrile:water 40:60, up; using scintillation detector.

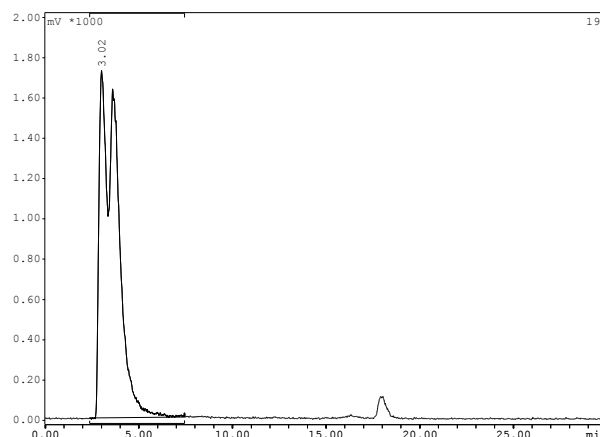


Fig 5. HPLC chromatograms of ^{177}Lu -ALN on a reversed phase column using acetonitrile:water 40:60, up; using scintillation detector.

Stability test was developed for the complex in presence of human serum at 37°C using ITLC as mentioned above and all data within 48 were above 89% at all time intervals.

The protein binding was to be 57% protein as determined by supernatant/debris counting of the serum-radiopharmaceutical mixture, while 43% are found in free form in the circulation. The protein

binding for the bisphosphonates ligand has been reported in different references from 54% in free form [11].

HA assay demonstrated high capacity binding for bisphosphonates to hydroxyapatite. Even at 5 mg amount of HA, more than 40% binding was observed, while at 10 mg HA used >90% of binding was obtained (Figure 6).

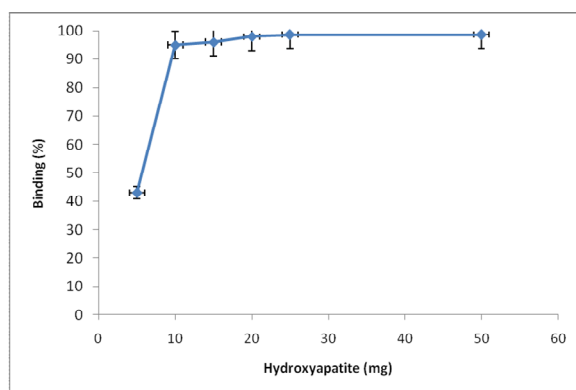


Fig 6. Hydroxy apatite binding assay for ^{177}Lu -ALN at 37°C in 24h.

In case of pamidronate however, the binding was weaker, at 10 mg amount of HA, more than 80% binding was observed, while at 20 mg HA used >90% of binding was obtained (Figure 7).

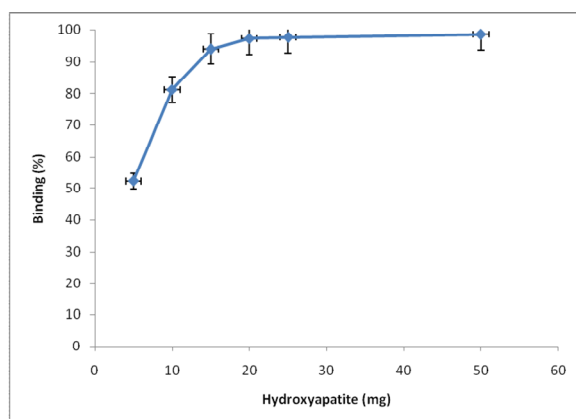


Fig 7. Hydroxy apatite binding assay for ^{177}Lu -PMD at 37°C in 24h.

With these findings, we went ahead with animal and biodistribution studies, although the data were weaker than ^{177}Lu -zoledronate as we have already reported.

In order to test the water/lipid solubility of the complexes, the partition coefficients were calculated

and as expected the radiolabeled complexes showed very polar characteristics possibly due to the anionic nature of the prepared complexes. The data is demonstrated in Table 1.

Table 1. The log P for the radiolabeled bisphosphonates.

Radiolabeled compound	log P
^{177}Lu -PMD	- 2.7
^{177}Lu -ALN	- 3.16

Biodistribution of ^{177}Lu cation and ^{177}Lu -bisphosphonates in wild-type rats

The animals were sacrificed by CO_2 asphyxiation at selected time points after tracer injection (2, 4, 24 and 48h). Dissection began by drawing blood from the aorta followed by removing the heart, spleen, muscle, bone, kidneys, liver, intestine, stomach, lungs and skin samples.

Regarding the animal studies, the tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g) (Figure 8, 9 and 10).

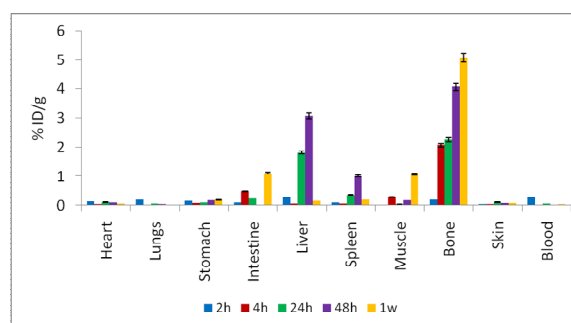


Fig 8. Percentage of injected dose per gram of $^{177}\text{LuCl}_3$ in wild type mice 24-7d post injection (n=3).

The biodistribution of ^{177}Lu cation was determined in wild-type animals for 2h-7d post injections. The liver radioactivity uptake of the cation is comparable to other radio-lanthanides such as Yb, Sm and Tb [14]. About 3 % of the cation radioactivity accumulates in the liver in 48 h. Low blood radioactivity content demonstrates the rapid removal of ^{177}Lu from the circulation after injection. Lung, muscle and skin do not demonstrate significant ^{177}Lu uptake while it is in accordance with other cations accumulation. A 4% bone uptake is observed for ^{177}Lu which remains almost constant after 7 h (5%). Spleen also has significant ^{177}Lu uptake possibly related to reticuloendothelial system.

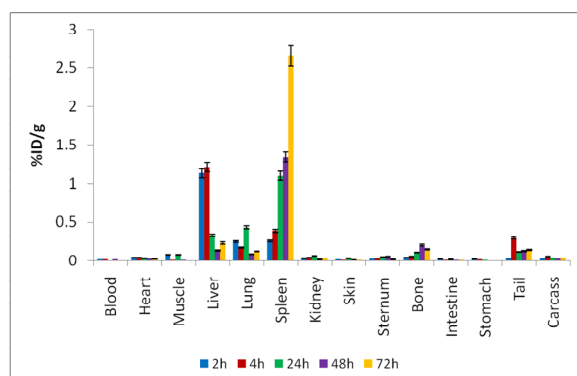


Fig 9. Percentage of injected dose per gram of ^{177}Lu -PMD in wild-type mice tissues at 2, 4, 24 and 48 h post injection (n=3).

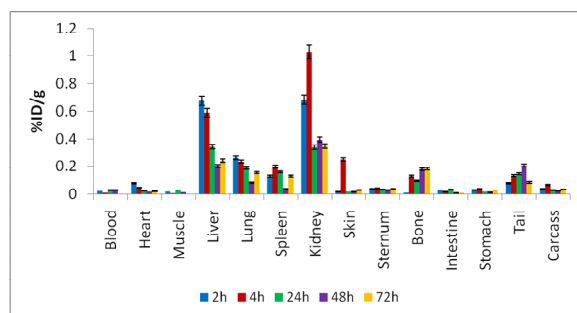


Fig 10. Percentage of injected dose per gram of ^{177}Lu -ALN in wild-type mice tissues at 2, 4, 24 and 48 h post injection (n=3).

The biodistribution of ^{177}Lu -PMD and ^{177}Lu -ALN in mice however, demonstrated very low bone uptake. The liver, lung and spleen uptake were much higher from other reported radiolabeled bisphosphonates. This data suggested the biological instability of the radiolabeled complexes possibly due to enzymatic reactions and/or other metabolic causes.

Our research demonstrated that, the direct radiolabeling of these compounds with lutetium do not yield effective agents for bone pain palliation candidacy. The idea of using conjugated chelates to the bis-phosphonate backbone is suggested.

CONCLUSION

^{177}Lu -bis-phosphonate was prepared (radiochemical purity >99%) using optimization studies. ^{177}Lu -bis-phosphonate and $^{177}\text{LuCl}_3$ preparations were administered intravenously through the tail vein to wild-type mice and biodistribution data was checked 2 h to 72 h later. The complexes were obtained in high radiochemical purity ITLC (>98%) and HPLC

(>98%). Satisfactory stability in presence of human serum and final formulations were obtained. HA binding assay demonstrated >95% binding from 5-20 mg of HA in 24h. The complex protein binding was about 55-58%. The high bone uptake ratios at all time intervals was in accordance with the HA test. However, in biodistribution studies low bone uptake ratios were observed, for both complexes far inferior to ^{177}Lu -EDTMP. The idea of using conjugated chelates to the bis-phosphonate backbone is to compensate for this shortcoming, suggested.

Acknowledgments

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REFERENCES

1. Serafini AN. Therapy of metastatic bone pain. *J Nucl Med.* 2001 Jun;42(6):895-906.
2. Campa JA 3rd, Payne R. The management of intractable bone pain: a clinician's perspective. *Semin Nucl Med.* 1992 Jan;22(1):3-10.
3. Eary JF, Collins C, Stabin M, Vernon C, Petersdorf S, Baker M, Hartnett S, Ferency S, Addison SJ, Appelbaum F, Gordon EE. Samarium-153-EDTMP biodistribution and dosimetry estimation. *J Nucl Med.* 1993 Jul;34(7):1031-6.
4. Yousefnia H, Jalilian AR, Zolghadri S, Ghannadi-Maragheh M. Production, quality control, biodistribution assessment and preliminary dose evaluation of (^{177}Lu)-PDTMP as a possible bone palliative agent. *Nucl Med Commun.* 2014 Jan;35(1):99-107.
5. Bahrami-Samani A, Anvari A, Jalilian AR, Shirvani-Arani S, Yousefnia H, Aghamiri MR, Ghannadi-Maragheh M. Production, Quality Control and Pharmacokinetic Studies of (^{177}Lu)-EDTMP for Human Bone Pain Palliation Therapy Trials. *Iran J Pharm Res.* 2012 Winter;11(1):137-44.
6. Alavi MS, Omidvari S, Mehdizadeh A, Gheisari F. Metastatic bone pain palliation using ^{177}Lu - EDTMP [abstract]. *Iran J Nucl Med.* 2012;20(Suppl 1):50.
7. Nikzad M, Jalilian AR, Shirvani-Arani S, Bahrami-Samani A, Golchoubian H. Production, quality control and pharmacokinetic studies of ^{177}Lu -zoledronate for bone pain palliation therapy. *J Radioanal Nucl Chem.* 2013;298(2):1273-1281.
8. Zolghadri S, Jalilian AR, Naseri Z, Yousefnia H, Bahrami-Samani A, Ghannadi-Maragheh M, Afarideh H. Production, quality control and biological evaluation of (^{166}Ho)-PDTMP as a possible bone palliation agent. *Iran J Basic Med Sci.* 2013 May;16(5):719-25.
9. Zolghadri S, Jalilian AR, Yousefnia H, Bahrami-Samani A, Fazaali Y, Pouladi M, Ghannadi-Maragheh M, Afarideh H. The synthesis, radiolabeling and first

- biological evaluation of a new ^{166}Ho -complex for radiotherapy of bone metastases. *Radiochimica Acta*. 2013;101(7):445-51.
10. International Atomic Energy Agency. Manual for reactor produced radioisotopes. Vienna: IAEA; 2003. p.121-23.
 11. EANM Radiopharmacy Committee, Guidelines on current good Radiopharmacy Practice (cGRPP) in the Preparation of Radiopharmaceuticals. cGRPP-guidelines, version 2, March 2007.
 12. Dar UK, Khan IU, Javed M, Ahmad F, Ali M, Hyder SW. Preparation and biodistribution in mice of a new radiopharmaceutical-technetium-99m labeled methotrexate, as a tumor diagnostic agent. *Hell J Nucl Med*. 2012 May-Aug;15(2):120-4.
 13. Neves M, Gano L, Pereira N, Costa MC, Costa MR, Chandia M, Rosado M, Fausto R. Synthesis, characterization and biodistribution of bisphosphonates Sm-153 complexes: correlation with molecular modeling interaction studies. *Nucl Med Biol*. 2002 Apr;29(3):329-38.
 14. Du XL, Zhang TL, Yuan L, Zhao YY, Li RC, Wang K, Yan SC, Zhang L, Sun H, Qian ZM. Complexation of ytterbium to human transferrin and its uptake by K562 cells. *Eur J Biochem*. 2002 Dec;269(24):6082-90.