Absorbed dose assessment of ^{177}Lu-zoledronate and ^{177}Lu-EDTMP for human based on biodistribution data in rats

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ABSTRACT

Over the past few decades, several bone-seeking radiopharmaceuticals including various bisphosphonate ligands and β-emitting radionuclides have been developed for bone pain palliation. Recently, ^{177}Lu was successfully labeled with zoledronic acid (^{177}Lu-ZLD) as a new generation potential bisphosphonate and demonstrated significant accumulation in bone tissue. In this work, the absorbed dose to each organ of human for ^{177}Lu-ZLD and ^{177}Lu-ethylenediaminetetramethylene phosphonic acid (^{177}Lu-EDTMP; as the only clinically bone pain palliation agent) was investigated based on biodistribution data in rats by medical internal radiation dosimetry (MIRD) method. ^{177}Lu-ZLD and ^{177}Lu-EDTMP were prepared in high radiochemical purity (>99%, instant thin layer chromatography (ITLC)) at the optimized condition. The biodistribution of the complexes demonstrated fast blood clearance and major accumulation in the bone tissue. The highest absorbed dose for both ^{177}Lu-ZLD and ^{177}Lu-EDTMP is observed in trabecular bone surface with 12.173 and 10.019 mSv/MBq, respectively. The results showed that ^{177}Lu-ZLD has better characteristics compared to ^{177}Lu-EDTMP and can be a good candidate for bone pain palliation.

Key words: ^{177}Lu, absorbed dose, biodistribution, EDTMP, zoledronate

Introduction

Metastatic bone cancer is a common and severe complication in advanced diseases.[1,2] Radionuclide therapy is shown to be useful and cost effective in relieving bone pain in metastatic diseases and may be more effective when combined with chemotherapy and the use of bisphosphonates.[3]

According to the results of three randomized phase III clinical trials enrolling more than 3,000 patients; zoledronic acid (1-hydroxy-2-(imidazol-1-yl-amino)-ethylidene-biphosphonic acid) [Figure 1] has showed its high potential as a new-generation bisphosphonate that is effective in the treatment of bone metastases secondary to all solid tumor types and bone lesions from multiple myeloma.[4-7]

Recently, ^{177}Lu was successfully labeled with zoledronic acid and its biodistribution was investigated in wild type rat studies, which demonstrated significant accumulation in bone tissue.[8] However, the absorbed dose to each human organ has not been reported to the best of the authors' knowledge. In this work, the absorbed dose to each human organ for ^{177}Lu-ZLD and ^{177}Lu-ethylenediaminetetramethylene phosphonic acid (EDTMP) [Figure 2] was evaluated based on biodistribution studies in rats by medical internal radiation dosimetry (MIRD) method.

Materials and Methods

^{177}Lu was produced by irradiation of natural Lu_2O_3 target at a thermal neutron flux of approximately 4×10^{13} n.cm^{-2}.s^{-1} for 5 days at Tehran Research Reactor (TRR). Zoledronic acid and sodium zoledronate were purchased from Sigma-Aldrich Co., UK. Whatman No. 3 paper was obtained from Whatman (UK). Radiochromatography was performed by using a Bioscan AR-2000 radio-TLC scanner instrument (Bioscan, France). Analytical high performance liquid

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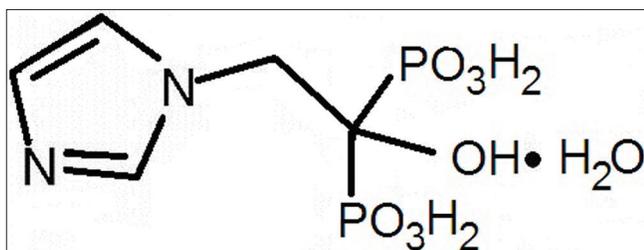
Figure 1: Chemical structure for zoledronic acid monohydrate

chromatography (HPLC) to determine the specific activity was performed by a Shimadzu LC-10AT, equipped with two detector systems, flow scintillation analyzer (Packard-150 TR), and ultra violet (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, USA) multichannel analyzer and a dose calibrator ISOMED 1010 (Elimpex-Medizintechnik, Austria) was used for counting distributed activity into rat 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 ± standard deviation and the percentage of the injected dose per gram (%ID/g) amounts for each rat organ was compared using Student's *t*-test. Statistical significance was defined as $P < 0.05$. Animal studies were carried out in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edition.[9] The approval of Nuclear Science and Technology Research Institute (NSTRI) Ethical Committee was obtained for conducting this research. The wild-type rats were purchased from Pasteur Institute of Iran, Karaj; all weighing 180–200 g and were acclimatized at proper rodent diet.

Production and quality control of [177]LuCl$_3$ solution

Lutetium-177 was produced by the neutron irradiation of 1 mg of natural Lu$_2$O$_3$ (99.999% from Aldrich Co, UK) according to the reported procedures[10] at TRR. The irradiated target was dissolved in 200 μL of 1.0 M HCl to prepare [177]LuCl$_3$ and diluted to the appropriate volume with ultrapure water to produce a stock solution of final volume of 5 mL (0.04 mol/L). The mixture was filtered through a 0.22 μm filter (Waters, USA) for sterilization. The radionuclidic purity of the solution was tested for the presence of other radionuclides using an HPGe detector for the detection of various interfering gamma-emitting radionuclides. The radiochemical purity of the [177]LuCl$_3$ was checked using two solvent systems for instant thin layer chromatography (ITLC) (A: 10 mmol.L - 1 diethylenetriaminepentaacetic acid (DTPA) at pH.5 and B: 10% ammonium acetate: methanol (1:1)).

Radiolabeling of ZLD with [177]LuCl$_3$

A stock solution of sodium zoledronate (molecular weight (MW) 334) was prepared by dissolution of the complex in
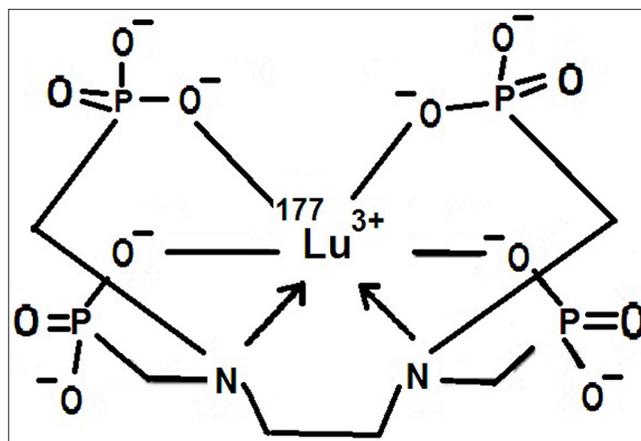
Figure 2: Chemical structure of ^{177}Lu-EDTMP. ^{177}Lu-EDTMP = ^{177}Lu-ethylenediaminetetramethylene phosphonic acid

double distilled ultrapure water, to produce a solution of 50 mg/mL. Radiolabeling of ZLD with [177]LuCl$_3$ was performed based on the previous literature.[8] Briefly, 5 mCi (185 MBq) of the [177]LuCl$_3$ solution (0.1 mL) was added to the desired amount of NaZLD solution (1:50 ratios for Lu: ZLD). The radiochemical purity was determined using ITLC(NH$_4$OH (56%):MeOH (100%):H$_2$O (100%) (0.2:2:4; v/v/v) as the mobile phase mixture) and HPLC method. The final solution was passed through a 0.22-μm membrane filter and pH was adjusted to 7–8.5 with 0.05 mol/L phosphate buffer (pH 5.5).

Radiolabeling of EDTMP with [177]LuCl$_3$

A stock solution of EDTMP was prepared by dissolving in 1 mol/L NaOH and diluted to the appropriate volume with ultrapure water, in order to produce a solution of 50 mg/mL. Radiolabeling of EDTMP with [177]LuCl$_3$ was performed based on the previous literature.[11] Briefly, 5 mCi (185 MBq) of the [177]LuCl$_3$ solution was added to the desired amount of EDTMP solution (0.3 mL, 1–5 mg/mL). The complex solution was then kept at room temperature for 60 min. The final solution was passed through a 0.22-μm membrane filter and the pH was adjusted to 7–8.5. The radiochemical purity was determined using Whatman No. 3 chromatography paper or ITLC-silica gel (SG), eluted with NH$_4$OH (56%):methanol (100%):water (100%) (0.2:2:4; v/v/v) mixture.

Biodistribution of ^{177}Lu-ZLD and ^{177}Lu-EDTMP in wild-type rats

The final complexes (100 μL including 3.7 MBq of radioactivity) were injected intravenously to the rats through their tail veins. The animals were sacrificed at the exact time intervals (2–168 h post injection), and the specific activity of the different organs was calculated as the %ID/g using an HPGe detector.

Dosimetric studies

The absorbed dose of each human organ was calculated by MIRD method based on biodistribution data in

wild-type rats. The accumulated activity in animals was extrapolated to the accumulated activity in humans by the proposed method of Sparks and Aydogan, (Equation 1).[12]

$$\tilde{A}_{human\ organ} = \tilde{A}_{animal\ organ} \frac{OrganMass_{human} / BodyMass_{human}}{OrganMass_{animal} / BodyMass_{animal}}$$

(1)

Where $\tilde{A}$ is the accumulated activity in the source organs and can be calculated by Equation 2.

$$\tilde{A} = \int_{t_1}^{\infty} A\ (t)\ dt$$

(2)

It should be noted that A (t) is the activity of each organ at time t.

The accumulated source activity for each organ of animals was calculated by plotting the percentage-injected dose versus time for each organ and computing the area under the curves. For this purpose the data points which represent the percentage-injected dose were created. Linear approximation was used between the two experimental time points. The curves were extrapolated to infinity by fitting the tail of each curve to a monoexponential curve with the exponential coefficient equal to physical decay constant of ^{177}Lu. Then the area under the curve was calculated. In order to extrapolate this accumulated activity to human, the mean weights of each organ for standard human were used.[13]

The radiation absorbed dose was calculated by MIRD formulation:

$$D = N \times DF$$

(3)

Where N is the number of disintegrations that occur in a source organ, and DF is:

$$DF = \frac{k \sum n\ E\ \phi}{m}$$

(4)

Where n is the number of radiations with energy E emitted per nuclear transition, E is the energy per radiation (MeV), ϕ is the fraction of energy emitted that is absorbed in the target, m is the mass of target region (kg), and k is some proportionality constant $\left(\frac{mGy.kg}{MBq.s.MeV}\right)$. DF represents the physical decay characteristics of the radionuclide, the range of the emitted radiations, and the organ size and configuration[14] expressed in mGy/MBq.s. DFs have been taken from the OLINDA/EXM software.[15]

Results

Radionuclide production

The radionuclide was prepared in the range of specific activity of 2.6–3 GBq.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 solvent systems. In 10 mmol/L DTPA aqueous solution (solvent 1), free Lu^{3+} cation was complexed to more lipophilic LuDTPA form and migrated to higher R_f. The small radioactive fraction which remained at the origin could be related to the other Lu ionic species, not forming LuDTPA complex, such as LuCl$_4^-$, and/or colloids. On the other hand, 10% ammonium acetate:methanol mixture (1:1) (solvent 2) was also used for the determination of radiochemical purity.

Radiolabeling of ZLD and EDTMP with ^{177}LuCl$_3$

ITLC studies approved the production of a single radiolabeled compound at the R_f 0.8, while Lu^{+3} retains to the lower R_f due to the polarity. The HPLC studies also demonstrated the existence of only one radiolabeled species using both UV and scintillation detectors.

Biodistribution of ^{177}Lu-ZLD and ^{177}Lu-EDTMP in wild-type rats

Three rats were sacrificed for each time interval. The tissue uptakes of the complex were calculated as %ID/g [Figures 3 and 4]. It is clearly shown that the major portion of the injected radioactivity of ^{177}Lu–ZLD and ^{177}Lu-EDTMP was transferred from the blood circulation into the bones.

Dosimetric studies

Preliminary dosimetric evaluation of the complexes in human organs was performed by MIRD method based on biodistribution data in rat organs. First, the area under the clearance curves for each organ was calculated. Then, the absorbed dose was calculated according to the S factors in OLINDA software. The estimated absorbed dose in each human organ after injection of ^{177}Lu-ZLD and ^{177}Lu-EDTMP is given in Table 1. Furthermore, trabecular bone surface to other tissue dose ratio as target/nontarget dose ratio for ^{177}Lu-ZLD and ^{177}Lu-EDTMP is given in Table 2.

Discussion

Successful radionuclide pain palliation therapy is based on selective concentration and prolonged retention of the radiopharmaceutical at the skeletal lesions, while bone marrow dose should be kept as low as possible.[16] For this purpose, β-particles of low energies are recommended.

Among the β-emitter radioisotopes, ^{177}Lu ($t_{1/2}$ = 6.73 d, E_β(max) = 497 keV, and Eγ = 112 keV (6.4%), 208 keV (11%)) has better characteristics. The significant advantage of utilizing ^{177}Lu is its β-particle energies which are adequately low, therefore the bone marrow suppression is minimum
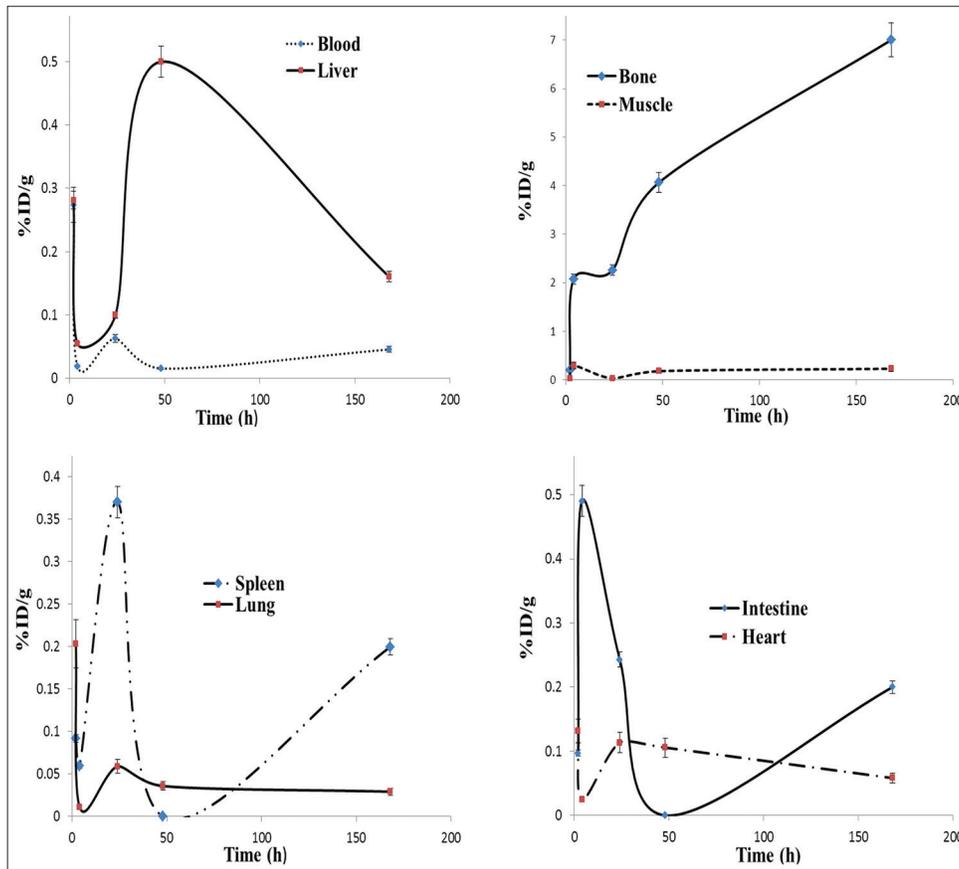
Figure 3: Percentage of injected dose per gram of ^{177}Lu-EDTMP in wild-type rat tissue after 2, 4, 24, 48, and 168 h post injection
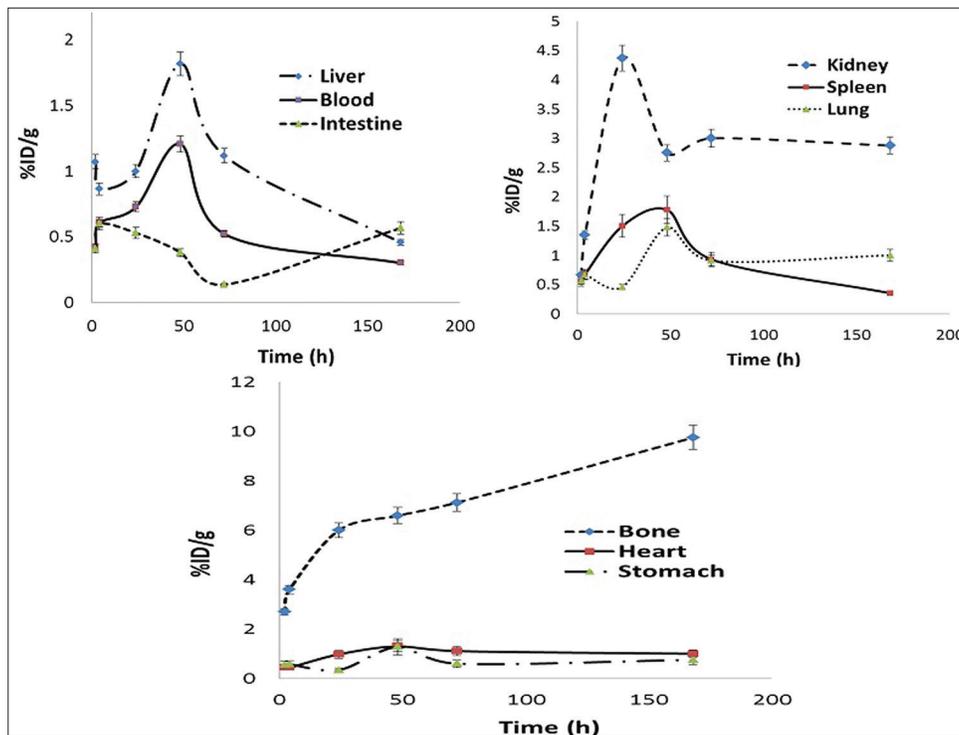
Figure 4: Percentage of injected dose per gram of ^{177}Lu-ZLD in wild-type rat tissue after 2, 4, 24, 48, and 168 h post injection. ^{177}Lu-ZLD = [177]Lu labeled with zoledronic acid

Table 1: The absorbed dose in each human organ after injection of ^{177}Lu-ZLD and ^{177}Lu-EDTMP

Tissue	Absorbed dose (mSv/MBq) ^{177}Lu-ZLD	Absorbed dose (mSv/MBq) ^{177}Lu-EDTMP	Tissue	Absorbed dose (mSv/MBq) ^{177}Lu-ZLD	Absorbed dose (mSv/MBq) ^{177}Lu-EDTMP
Adrenals	0.055±0.003	0.045±0.006	Ovaries	0.031±0.002	0.030±0.002
Brain	0.056±0.002	0.047±0.006	Pancreas	0.035±0.002	0.029±0.001
Breasts	0.016±0.001	0.013±0.001	Red marrow	3.997±0.407	3.291±0.247
Gallbladder wall	0.026±0.001	0.021±0.001	Cortical bone surface	9.524±0.803	7.839±0.655
Lower large intestine wall	0.419±0.006	0.217±0.005	Trabecular bone surface	12.173±1.018	10.019±0.714
Small intestine	0.028±0.001	0.025±0.001	Cortical bone volume	2.270±0.209	1.870±0.172
Stomach	0.103±0.005	0.018±0.001	Trabecular bone volume	5.850±0.627	4.816±0.416
Upper large intestine wall	0.024±0.001	0.022±0.002	Spleen	0.242±0.016	0.085±0.003
Heart content	0.172±0.007	0.040±0.003	Testes	0.017±0.001	0.017±0.001
Heart wall	0.430±0.009	0.065±0.003	Thymus	0.024±0.001	0.022±0.001
Kidneys	0.871±0.009	0.028±0.001	Thyroid	0.035±0.002	0.033±0.001
Liver	0.305±0.007	0.136±0.006	Urinary bladder wall	0.018±0.001	0.019±0.001
Lungs	0.391±0.008	0.037±0.002	Uterus	0.023±0.001	0.023±0.001
Muscle	0.035±0.002	0.134±0.007	Total body	0.794±0.022	0.683±0.008

^{177}Lu-ZLD: [177]Lu labeled with zoledronic acid, ^{177}Lu-EDTMP: ^{177}Lu-ethylenediaminetetramethylene phosphonic acid

Table 2: Trabecular bone surface to other tissue dose ratio for ^{177}Lu-ZLD and ^{177}Lu-EDTMP

Tissue	^{177}Lu-ZLD	^{177}Lu-EDTMP	Tissue	^{177}Lu-ZLD	^{177}Lu-EDTMP
Adrenals	218.274	219.881	Ovaries	381.847	331.508
Brain	216.389	213.158	Pancreas	340.012	337.002
Breasts	750.460	742.714	Red marrow	3.045	3.044
Gallbladder wall	466.595	457.080	Cortical bone surface	1.278	1.278
Lower large intestine wall	29.005	46.030	Trabecular bone surface	1.000	1.000
Small intestine	432.846	389.973	Cortical bone volume	5.360	5.356
Stomach	117.534	536.631	Trabecular bone volume	2.080	2.080
Upper large intestine wall	497.356	446.285	Spleen	50.254	116.886
Heart Content	70.586	249.520	Testes	685.637	560.163
Heart wall	28.280	153.742	Thymus	487.396	451.379
Kidneys	13.968	355.481	Thyroid	338.755	302.149
Liver	39.791	73.276	Urinary bladder wall	649.830	515.253
Lungs	31.127	267.727	Uterus	523.113	426.971
Muscle	347.315	74.553	Total body	15.316	14.666

^{177}Lu-ZLD: [177]Lu labeled with zoledronic acid, ^{177}Lu-EDTMP: ^{177}Lu-ethylenediaminetetramethylene phosphonic acid

when it accumulates in skeletal lesions.[17,18] Therefore, different bisphosphonates with ^{177}Lu have been developed and used for the bone pain palliation.

It has been proved that zoledronic acid as an osteoclast-mediated bone resorption inhibitor can be more potent than other bisphosphonates.[5] Zoledronic acid was 850-fold more effective than pamidronate at inhibiting the induction of hypercalcemia in rats, and 40- to 100-fold more potent.[5] Also, it is more than four orders of magnitude more potent than clodronate.[7]

Specific uptake and retention in the target organ and rapid clearance from nontarget organs are significant parameters for the each radiolabelled complex. Low blood accumulation for both ^{177}Lu-ZLD and ^{177}Lu-EDTMP (<1.5%) demonstrated rapid washout from blood circulation. Besides, accumulation of both compounds in the bone increases with time up to 168 h which shows long-term retention of the complexes in the target organ.

There are two major sources of toxicity from these radiolabeled complexes: Unchelated lutetium and the accumulation of the radiolabeled complexes in the nontarget organs. Lu^{3+} metal has been found to distribute to the liver, spleen, and kidneys.[19] Thus, it is necessary to keep as little unchelated Lu^{3+} as possible to avoid uptake by liver and hepatotoxicity. Additionally, bone marrow toxicity is the most important point which should be considered for bone avid radiopharmaceuticals. While the absorbed dose of bone marrow is approximately identical for both ^{177}Lu-ZLD and ^{177}Lu-EDTMP, both of these complexes would have the same toxicity in bone marrow.

The effects of radionuclides in the management of disease are often estimated by the absorbed dose to target

organ relative to normal tissues.[20] A prerequisite for the clinical application of a new radiopharmaceutical is the measurement of organ radiation exposure dose from biodistribution data in animals.[21] Calculation of the radiopharmaceutical's absorbed dose in human organs from biodistribution in small animals can be useful for determining the injected activity and accelerating the development of radioactive compounds to be used in clinical settings and is a common first step consistent with the recommendations of International Commission on Radiological Protection (ICRP) 62.[22]

Recently, the ^{177}Lu-ZLD complex has suggested as a promising radiolabeled compound for targeted bone pain palliation. In this work, the absorbed dose to each organ of human for ^{177}Lu-ZLD was evaluated based on biodistribution studies in rats by MIRD method. Extrapolation between animal data to human may lead to some over- or underestimation, previous studies have indicated the usefulness of using animal biodistribution as a model for absorbed dose estimations in humans.[23]

The only clinically used Lu-177 bone pain palliation therapeutic agent is ^{177}Lu-EDTMP, which employed in early clinical trials in some centers in the world.[24,25] Consequently, for preliminary comparison of ^{177}Lu-ZLD as a bone pain palliation agent, the absorbed dose to each human organ for this complex is compared to ^{177}Lu-EDTMP [Table 1]. As expected, the highest absorbed dose for both [177]Lu- ZLD and ^{177}Lu-EDTMP is observed in trabecular bone surface with 12.173 and 10.019 mSv/MBq, respectively.

The importance of an ideal therapeutic radiopharmaceutical relays on the ratio of the absorbed dose in target organ to other organs. Therefore, trabecular bone surface to other tissue dose ratio for ^{177}Lu-ZLD and ^{177}Lu-EDTMP was calculated. While, the dose ratio of trabecular bone surface to the most tissue for ^{177}Lu-ZLD is in the same order or better in some cases than for ^{177}Lu-EDTMP, the trabecular bone surface to heart, kidney, liver, lung, and spleen dose ratio is considerably higher for ^{177}Lu-EDTMP compared to ^{177}Lu-ZLD, which puts ^{177}Lu-ZLD in disadvantageous situation compared to ^{177}Lu-EDTMP. In comparison to ^{177}Lu-EDTMP, the most important advantage of ^{177}Lu-ZLD is the dose delivered in other organs such as breast, intestine, testes thymus, thyroid urinary bladder, uterus, and even total body. Also, muscle uptake is considerably lower for ^{177}Lu-ZLD which can improve the quality of single-photon emission computed tomography (SPECT) images; therefore, the treatment can follow with better precision.

Conclusion

In this study, ^{177}Lu-ZLD and ^{177}Lu-EDTMP complexes were prepared in high radiochemical purity (>99%,

ITLC) at the optimized condition. Satisfactory stability in presence of human serum and final formulations was obtained for both of the complexes. The biodistribution of the complexes was checked up to 168 h post injection, showing fast blood clearance and major accumulation in the bone tissue. The absorbed dose to each organ of human for ^{177}Lu-ZLD and ^{177}Lu-EDTMP was evaluated based on biodistribution studies in rats by MIRD method. The highest absorbed dose for both [177]Lu- ZLD and ^{177}Lu-EDTMP is observed in trabecular bone surface with 12.173 and 10.019 mSv/MBq, respectively. In comparison to ^{177}Lu-EDTMP, the most important advantage of ^{177}Lu-ZLD is the dose delivered in the critical organs. Also, muscle uptake is considerably lower for ^{177}Lu-ZLD which can improve the quality of SPECT images, therefore, the treatment can follow with better precision. ^{177}Lu-ZLD complex demonstrated better characteristics compared to ^{177}Lu-EDTMP, and therefore can be a good candidate for bone pain palliation.

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