

Production, biodistribution assessment and dosimetric evaluation of ^{177}Lu -TTHMP as an agent for bone pain palliation

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ABSTRACT

Objective(s): Recently, bone-avid radiopharmaceuticals have been shown to have potential benefits for the treatment of widespread bone metastases. Although ^{177}Lu -triethylene tetramine hexa methylene phosphonic acid (abbreviated as ^{177}Lu -TTHMP), as an agent for bone pain palliation, has been evaluated in previous studies, there are large discrepancies between the obtained results. In this study, production, quality control, biodistribution, and dose evaluation of ^{177}Lu -TTHMP have been investigated and compared with the previously reported data.

Methods: TTHMP was synthesized and characterized, using spectroscopic methods. Radiochemical purity of the ^{177}Lu -TTHMP complex was determined using instant thin-layer chromatography (ITLC) and high performance liquid chromatography (HPLC) methods. The complex was injected to wild-type rats and biodistribution was studied for 7 days. Preliminary dose evaluation was investigated based on biodistribution data in rats.

Results: ^{177}Lu was prepared with 2.6-3 GBq/mg specific activity and radionuclide purity of 99.98%. ^{177}Lu -TTHMP was successfully prepared with high radiochemical purity (>99%). The complex showed rapid bone uptake, while accumulation in other organs was insignificant. Dosimetric results showed that all tissues received almost insignificant absorbed doses in comparison with bone tissues.

Conclusion: Based on the obtained results, this radiopharmaceutical can be a good candidate for bone pain palliation therapy in skeletal metastases.

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Introduction

Bone metastasis is a common and severe complication in advanced stages of cancer (1, 2). It develops in up to 70% of patients with prostate cancer and breast cancer, and in nearly 30% of those with lung, bladder, and thyroid cancers (3, 4).

Standard treatments for this condition include systemic therapies (e.g., analgesics, chemotherapy, hormonal therapy, and bisphosphonates) and local control (e.g., radiation therapy, radiofrequency ablation, and surgical stabilization of the affected site) (5).

The proven efficacy and minimal toxicity of radiation therapy make it very suitable for pain palliation in cancer patients (3). Radionuclide

therapy, by using specific tumor-seeking radiopharmaceuticals, can be applied as a treatment for bone metastases. Recently, various phosphonate ligands, labeled with β -emitting radionuclides, have been effective in metastatic bone pain palliation (6, 7).

Successful bone pain palliation is based on selective concentration and prolonged retention of the radiopharmaceutical at skeletal lesions, while keeping the bone marrow absorbed dose as low as possible (8); for this purpose, low energy β -particles are recommended.

Recently, radiopharmaceuticals with radionuclides such as ^{32}P , ^{89}Sr , ^{186}Re , ^{188}Re , ^{153}Sm , ^{166}Ho , and ^{177}Lu have been developed for the

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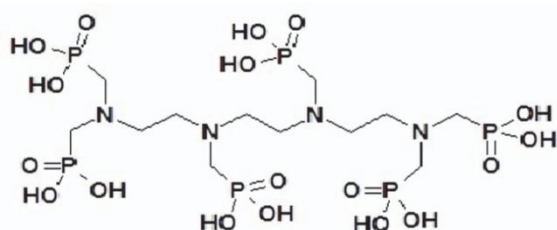


Figure 1. The chemical structure of TTHMP

treatment of painful metastases. Among these radioisotopes, ¹⁷⁷Lu [$t_{1/2}$ =6.73 d, $E_{\beta}(\text{max}) = 497$ keV, E_{γ} =112 keV (6.4%), 208 keV (11%)] has notable characteristics. The significant advantage of utilizing ¹⁷⁷Lu is its β -particle energies which are adequately low; therefore, bone marrow suppression is minimum when it accumulates in skeletal lesions (9, 10). Various bone-avid agents with ¹⁷⁷Lu have been developed and used for pain palliation including ¹⁷⁷Lu-DOTMP (11) and ¹⁷⁷Lu-EDTMP (12).

Improved cancer treatments and increased emphasis on the quality of life of cancer patients have led to a search for effective pain palliation for clinically active bone metastases, with fewer short-term and long-term side-effects. Therefore, various ¹⁷⁷Lu complexes including PYP (13), DTPMP, TTHMP (14), MDP (15), HEDP (16), and CTMP (17) have been developed and evaluated.

Among these radiopharmaceuticals, ¹⁷⁷Lu-triethylene tetramine hexa methylene phosphonic acid (abbreviated as ¹⁷⁷Lu-TTHMP) has been shown to have favorable characteristics. This complex accumulates massively in the bone, while its accumulation in other critical organs is very slow. Previous studies on ¹⁷⁷Lu-TTHMP have shown that this complex is comparable to other radiopharmaceutical complexes such as ¹⁸⁸Re-HEDP and ¹⁷⁷Lu-EDTMP, used in clinical trials; however, the results of these studies are inconsistent. Furthermore, despite the direct relationship between the absorbed dose and the effect of radiopharmaceuticals in disease management, the absorbed dose of this complex has not been reported, so far.

Accordingly, in this study, TTHMP was synthesized and labeled with ¹⁷⁷Lu. The chemical structure of the ligand is demonstrated in Figure 1. The parameters affecting radiochemical purity such as ligand concentration, pH of the reaction mixture, and incubation time were investigated. Biodistribution of the complex in wild-type rats was surveyed for better comparison with the reported studies. Finally, the preliminary dose evaluation in different human organs was investigated based on distribution data in rats.

Methods

¹⁷⁷Lu was produced by irradiation of natural Lu₂O₃ target at a thermal neutron flux of approximately 4×10^{13} n/cm²/s for 5 days at Tehran Research Reactor (TRR). Whatman No. 3 paper was obtained from Whatman, UK.

Radiochromatography was performed using a Bioscan AR-2000 radio-TLC scanner (Bioscan, France). Analytical high performance liquid chromatography (HPLC) method was applied to determine specific activity of the complex by a Shimadzu LC-10AT, equipped with two detector systems, flow scintillation analyzer (Packard-150 TR), and UV-visible spectrophotometer (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™ multichannel analyzer (model GC1020-7500SL, Canberra Industries Inc., CT, U.S.A.) and a dose calibrator ISOMED 1010 (Elimpex-Medizintechnik, Austria) was used for measuring the distributed activity in rat organs. All other chemical reagents were purchased from Merck, Germany.

Calculations were based on the 112 keV peak for ¹⁷⁷Lu. All values were expressed as mean \pm standard deviation, and the data were compared using Student's T-test. 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). The approval of NSTRI Ethical Committee was obtained for conducting this research.

The wild-type rats (NMRI), weighing 180-200g, were purchased from Pasteur Institute of Karaj, Iran, and were acclimatized to proper rodent diet.

Production and quality control of ¹⁷⁷LuCl₃ solution

Lutetium-177 was produced by neutron irradiation of 1 mg of natural Lu₂O₃ (99.999% from Aldrich Co., UK), according to the reported procedures (18) at Tehran Research Reactor (TRR). The irradiated target was dissolved in 200 μ L of 1.0 M hydrochloric acid (HCl) to prepare ¹⁷⁷LuCl₃; then, it was diluted to the appropriate volume with ultrapure water to produce a stock solution with the final volume of 5 ml (0.04 mol/l).

The mixture was filtered through a 0.22 μ m filter for sterilization (Waters, U.S.A). The radionuclidic purity of the solution was tested for the presence of other radionuclides, using HPGe spectroscopy for the detection of various

interfering gamma-emitting radionuclides. The radiochemical purity of ¹⁷⁷LuCl₃ was checked using 2-solvent systems for instant thin-layer chromatography (ITLC) [A: 10 mmol/l diethylene triamine pentaacetic acid (DTPA) at pH 5 and B: 10% ammonium acetate-methanol (1:1)].

Synthesis of TTHMP

The experimental procedure for the synthesis of TTHMP ligand was in accordance with other bisphosphonates, as reported (14). Briefly, a quantity of 0.48 g (3.3 mM) of triethylenetetramine was dissolved in 0.75 ml of concentrated HCl and a concentrated aqueous solution of 1.62 g phosphorous acid (20 mM). The resulting solution was heated to the reflux temperature and 3.2 ml of 37% aqueous formaldehyde solution (40 mM) was added dropwise during 1 h to the refluxing solution, and the refluxing was continued for another 1 h. The result of the reaction was a precipitated ethanol of a white product from the concentrated reaction solution.

Radio-labeling of TTHMP with ¹⁷⁷LuCl₃

A stock solution of TTHMP was prepared by dissolution in 1 N NaOH) and was diluted to the appropriate volume with ultrapure water through dissolving a specific amount of ligand in 1.5 ml NaOH (2 N) and 3.5 ml distilled H₂O (pH=12). Then, 0.3 ml of this solution was added to 200 μl ¹⁷⁷LuCl₃ (210.9 MBq) and pH was adjusted to 7, using a phosphate buffer.

The reaction mixtures were incubated by stirring at room temperature for 1 h. Various parameters such as ligand concentration, pH of the reaction mixture, and incubation time were optimized to achieve maximum complexation yield. The radiolabeling yield of the ligand was determined with paper chromatography, using Whatman No. 3 paper by sampling 5 μl of the reaction mixture on the paper strip, developed in NH₄OH:MeOH:H₂O (1:10:20) mixture.

Stability studies

Stability of the complexes stored at room temperature (22°C) and in human serum (37°C) was studied at different intervals by determining the radiochemical purity of complexes, using paper chromatography in NH₄OH:MeOH:H₂O (1:10:20) system.

In vitro protein binding of ¹⁷⁷Lu-TTHMP in presence of human serum

In vitro protein binding of the complex was carried out in human blood by protein

precipitation. One ml of the labeled complex was mixed with 3 ml of fresh human plasma, and incubated for 1 h at 37°C. Content of the tube was centrifuged at 3000 rpm for 10 min in order to separate the serum from blood cells. After mixing an 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 a fraction of protein-bound radioactivity in percentage of total radioactivity.

Biodistribution of ¹⁷⁷Lu-TTHMP in wild-type rats

Final ¹⁷⁷Lu-TTHMP solution of 3.7 MBq in 50-100 μl was injected intravenously to the rats through their tail vein. The animals were sacrificed at specified time intervals (2, 4, 24, 48, 72, and 168 h), and specific activity of different organs was calculated as the percentage of injected dose per gram (%ID/g), using HPGe detector.

Dosimetric studies

The absorbed dose of each human organ was calculated by medical internal radiation dosimetry (MIRD) method, based on biodistribution data in wild-type rats. For this purpose, first the accumulated source activity was calculated by plotting the clearance curves for each organ and computing the area under the curves. Then, the accumulated activity in animals was extrapolated to the accumulated activity in humans by the proposed method of Sparks et al. (equation 1) (19).

$$\dot{A}_{\text{human organ}} = \dot{A}_{\text{animal organ}} \times \frac{\text{OrganMass}_{\text{human}} / \text{BodyMass}_{\text{human}}}{\text{OrganMass}_{\text{animal}} / \text{BodyMass}_{\text{animal}}} \quad (1)$$

In order to extrapolate the accumulated activity to human, the mean weight of each organ for standard human were considered. Finally, the absorbed radiation dose was calculated by MIRD formulation (20):

$$D(r_k) = \sum_h \dot{A}_h \times S(r_k \leftarrow r_h) \quad (2)$$

Where D(r_k) is the absorbed dose of the target organ and S(r_k) is defined as the mean absorbed dose to the target region r_k per unit accumulated activity in the source region r_h. The S factors have been obtained from OLINDA software (21).

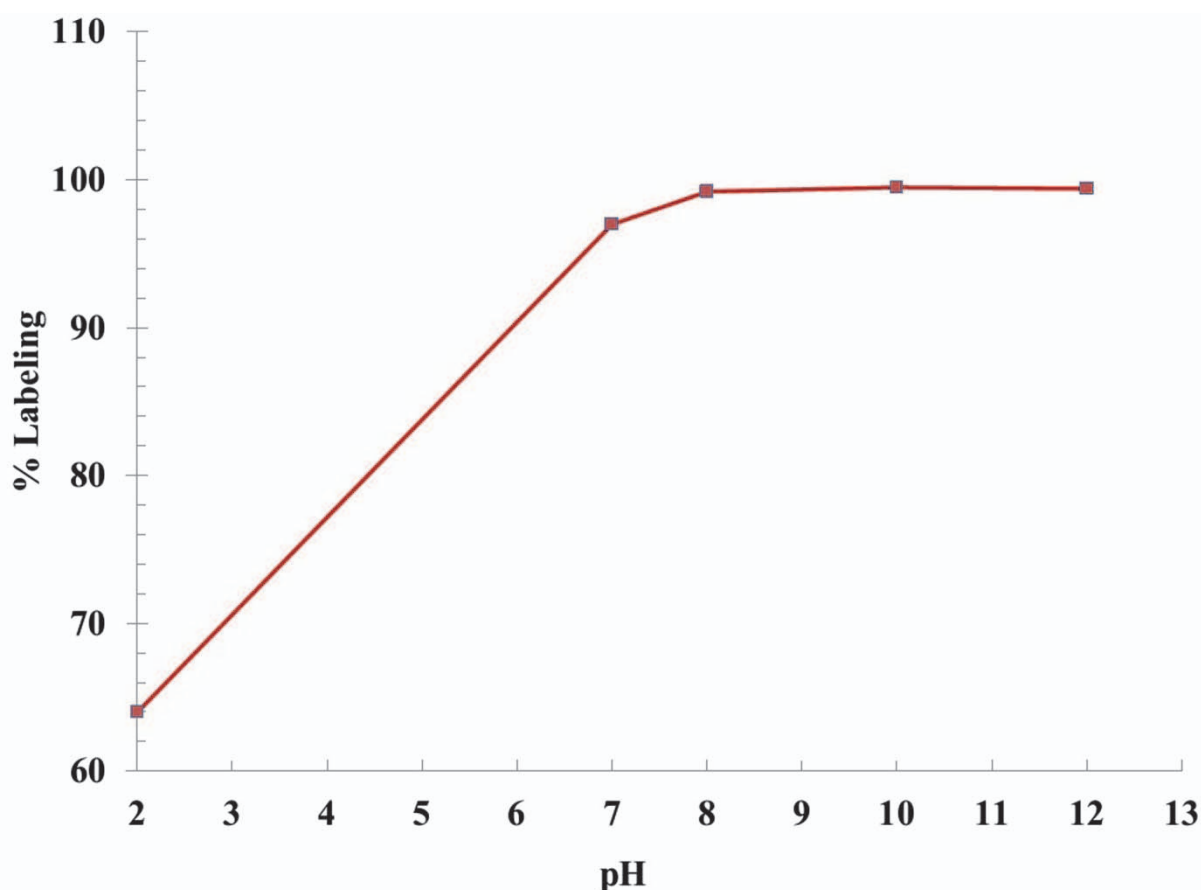


Figure 2. Effect of pH variation on complexation yield of ¹⁷⁷Lu-TTHMP at room temperature

Results

Radionuclide production

The radionuclide was prepared in the range of 2.6-3 GBq/mg specific activity for radiolabeling use. After counting the samples on the 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 ¹⁷⁷Lu solution was checked in the 2 solvents. In 10 mmol/l DTPA aqueous solution (solvent 1), free Lu³⁺ cation was complexed to more lipophilic Lu-DTPA form and migrated to higher R_f. The small radioactive fraction, which remained at the origin, could be related to other Lu ionic species, which were not involved in forming Lu-DTPA complex, such as LuCl₄⁻ and/or colloids. On the other hand, 10% ammonium acetate-methanol mixture (1:1) (solvent 2) was used for the determination of radiochemical purity.

Ligand synthesis

The TTHMP ligand was synthesized and its structure was determined using Proton Nuclear Magnetic Resonance (¹H NMR), mass, and infrared (IR) methods.

TTHMP: [m.p. 90-92°C, ¹H-NMR (D₂O, δ [ppm]): 3.02-3.25(m, 12 H, >N-CH₂CH₂-N<), 3.37-3.47(m, 12 H, -NCH₂-PO₃H₂)].

Mass: m/z (%), 710 (M+, % 26), 370 (%55), 233 (%44), 116 (%63), 110 (%100).

Labeling optimization studies

In order to obtain maximum complexation yields, several experiments were carried out by various reaction parameters such as ligand concentration, pH, and reaction time. The effect of pH variation on complexation yield of all ligands at room temperature was also studied by varying the pH of reaction mixture from 2 to 12, using 1 M HCl or 2 M NaOH solution. Maximum yield of 99% was observed at pH=7-8 for complexes. The effect of pH on complexation yield is shown in Figure 2.

Ligand concentration was varied within a wide range of 10 to 50 mg/ml for each ligand. It was observed that at room temperature, complexation >99% was achieved with 50:1 ligand-molar ratio.

The reaction mixture was incubated at room temperature for different time periods and 60 min incubation was found to be adequate to

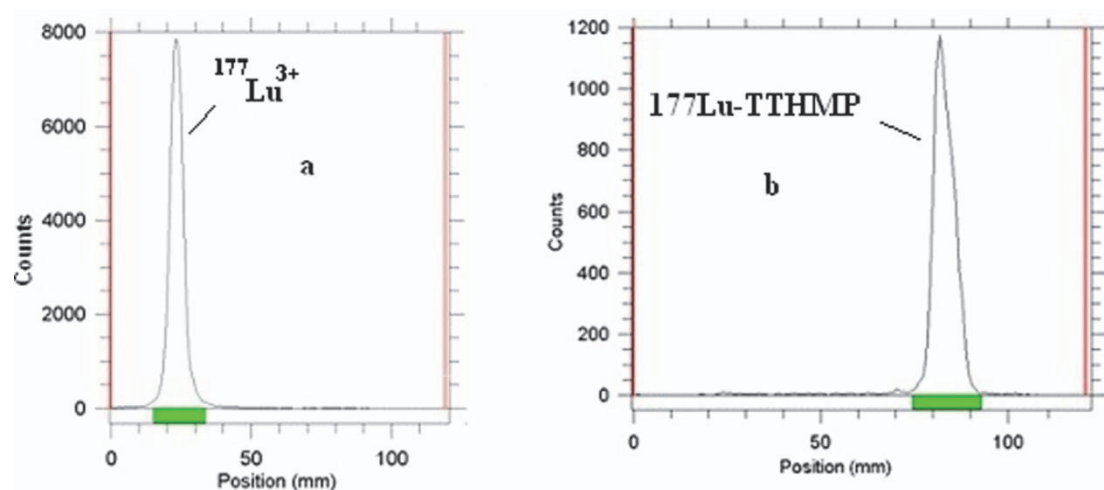


Figure 3. (a) ITLC chromatograms of $^{177}\text{LuCl}_3$ solution, and (b) $^{177}\text{Lu-TTHMP}$ in a $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (0.2:2:4) solution, using Whatman 3 MM

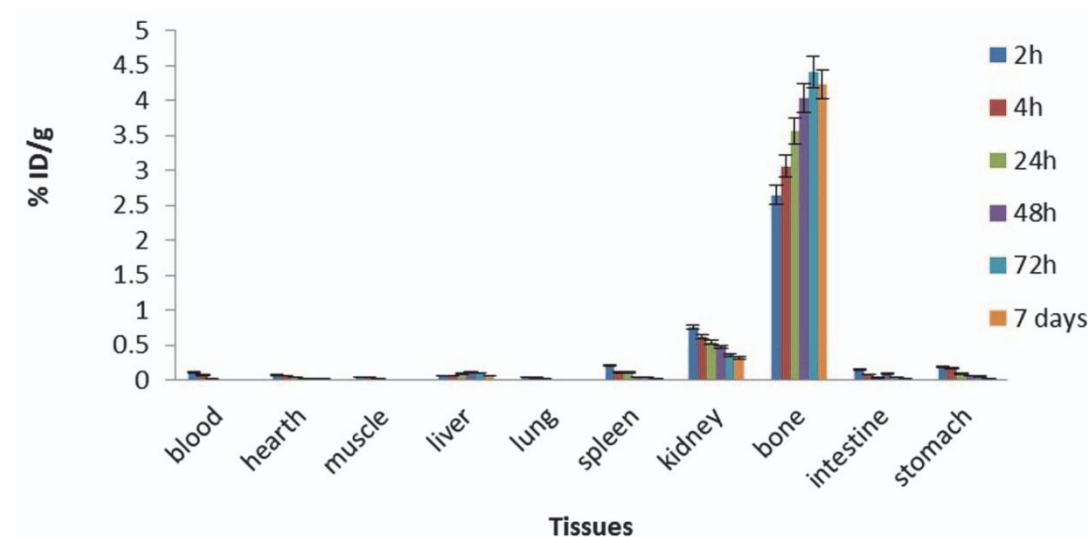


Figure 4. Biodistribution of $^{177}\text{Lu-TTHMP}$ in different organs of wild-type rats

yield maximum complexation. The best ITLC mobile phase was Whatman 3 MM paper, using $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (1:10:20), as shown in Figure 3.

Although the ITLC studies confirmed the production of the radiolabeled compound, HPLC studies demonstrated the existence of one radiolabeled species, utilizing both UV and scintillation detectors.

Stability studies

The stability of $^{177}\text{Lu-TTHMP}$, prepared under optimized reaction conditions, was studied, and the complex showed excellent stability both in human serum at 37°C and at room temperature after 72 h.

In vitro protein binding of the complex was carried out in human blood by protein precipitation. Protein binding of the complex was expressed as a fraction of protein-bound

radioactivity in percentage of total radioactivity. The complex protein binding was approximately 58–60%.

Biodistribution of $^{177}\text{Lu-TTHMP}$ in wild-type rats

The tissue uptake of the complex was calculated as the percentage of area under the curve of the related photopeak per gram of tissue (% ID/g) (Figure 4). The major radioactivity was accumulated in bones, as expected for bone-avid radiopharmaceuticals; the complex was also excreted through the kidneys.

Dosimetric studies

Preliminary dosimetric evaluation in human organs was performed by MIRD method, based on biodistribution data in rat organs. First, the area under the clearance curve was calculated

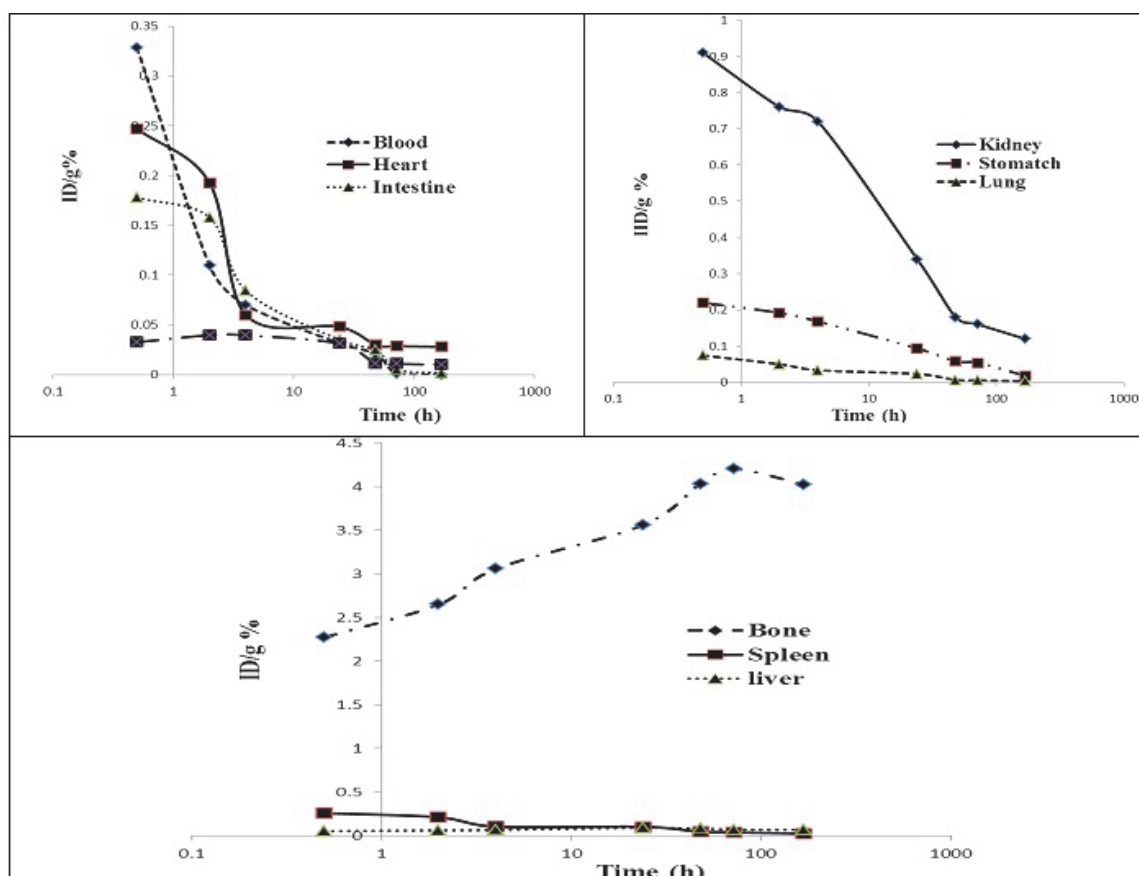


Figure 5. The clearance curves of each organ of rats

Table 1. Absorbed dose in each organ after the injection of ¹⁷⁷Lu-TTHMP

Organ	Absorbed dose (mSv/MBq)	Organ	Absorbed dose (mSv/MBq)
Adrenal glands	0.032	Ovaries	0.019
Brain	0.036	Pancreas	0.020
Breasts	0.009	Red Mar.	2.538
GB Cont.	0.013	Cort Bone Sur.	6.049
LLI Cont.	0.035	Trab. Bone Sur.	7.731
SI Cont.	0.017	Cort Bone Vol.	1.441
Stom. Cont.	0.018	Trab. Bone Vol.	3.716
ULI Cont.	0.014	Spleen	0.031
Heart Cont.	0.023	Testes	0.011
Heart Wall	0.034	Thymus	0.014
Kidneys	0.114	Thyroid	0.023
Liver	0.048	UB Cont	0.011
Lungs	0.022	Uterus	0.014
Muscle	0.028	Tot. Body	0.496

for each organ (Figure 5). Then, the absorbed dose was calculated according to the *s* factors in OLINDA software. The absorbed doses in each organ after ¹⁷⁷Lu-TTHMP injection are shown in Table 1.

Discussion

The radiolabeled ¹⁷⁷Lu-TTHMP was prepared with high radiochemical purity (>99%, ITLC) and by specific activity of 2.6-3 GBq/mg. ¹⁷⁷Lu-TTHMP demonstrated a great stability at room

temperature. The final product was administered to wild-type rats and biodistribution of the radiopharmaceutical was checked 2-168 h later. The results showed massive accumulation in the bone tissue, which increased up to 7 days (4.22 %ID/g). The complex was rapidly cleared of blood and no significant uptake was observed in critical organs.

¹⁷⁷Lu-TTHMP has been prepared and reported in previous studies (14, 16). In these studies, significant accumulation was reported in the bone, while accumulation in other organs was negligible. However, in this study, the complex pharmacokinetics in the bone followed a pattern similar to previously reported research, although the amount of radioactivity in the bone (%ID/g) was considerably different.

Lungu et al. investigated the biodistribution of ¹⁷⁷Lu-TTHMP in rats, demonstrating a rapid uptake in bone 4 h after the injection (79 %ID/g). Accumulation in the bone increased until 72 h after the injection and reached about 93 %ID/g. In the study by Chakraborty et al., the bone uptake was reported as 6.58 %ID/g, 3 h, which increased up to 7.82 %ID/g 96 h after the injection. In the current study, the maximum bone uptake of 4.40 %ID/g was obtained 72 h

Table 2. Optimum conditions for radiolabeling of ¹⁷⁷Lu-TTHMP

Reference	The current study	Ref. 14	Ref. 16
Ligand/metal	50:1	60:1	-
pH	7-8	9	7-7.5
Temperature	Room temperature	Room temperature	90°C
Time	60 min	15 min	90 min
Radiochemical purity	>99%	97.7%	>98%

Table 3. Comparison of liver, kidney, and spleen uptake for ¹⁷⁷Lu-TTHMP complex in normal rats (%ID/g)

	2 h	3 h	4 h	24 h	48 h	References
Liver	0.06	-	0.06	0.09	0.10	This study
	-	0.15	-	0.13	0.13	Ref. 14
Kidney	0.76	-	0.62	0.54	0.48	This study
	-	0.48	-	0.37	0.36	Ref. 14
Spleen	0.21	-	0.11	0.11	0.04	This study
	-	0.38	-	0.19	0.26	Ref. 14

after the injection, which is in agreement with the findings of Chakraborty and colleagues (14).

According to Mitterhauser’s findings (22), radiopharmaceutical preparation conditions strongly influence bone binding. Therefore, the differences in bone accumulation of ¹⁷⁷Lu-TTHMP, reported in various studies, may be due to variations in preparation conditions of the complex. The optimum conditions for radiolabeling of the complex in this research and previous studies are provided in Table 2.

In addition to the bone, low accumulations were observed in the liver, kidney, and spleen. The amount of radioactivity (%ID/g) in these tissues is demonstrated in Table 3. According to the results of Chakraborty et al., maximum liver uptake (0.15 %ID/g) was reported 3 h after the injection, while the best liver uptake was obtained after 48 h in the current study (0.10 %ID/g). Except the liver uptake, the pharmacokinetics of the complex in kidney and spleen followed a similar pattern, demonstrating the uptake reduction of kidney and spleen with time.

In this study, the absorbed dose of the complex was investigated for the first time. As expected, the highest absorbed dose for this complex was observed in the bone surface with 7.731 mSv/MBq.

Conclusion

According to the results, ¹⁷⁷Lu-TTHMP massively accumulated in the bone, while no significant accumulation was observed in other organs. Also, the dosimetric results demonstrated that the bone surface/red marrow dose ratio was approximately 3, while this ratio was 1.5 for ¹⁶⁶Ho-DOTMP (6). Considering these desirable characteristics, this radiopharmaceutical can be a good candidate for bone pain palliation therapy in skeletal metastases.

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