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Preliminary Dosimetric Evaluation of a New Therapeutic ^{177}Lu Complex for Human Based on Biodistribution Data in Rats

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***Abstract*—**[Tris (1,10-phenanthroline) lanthanum(III)] trithiocyanate is a new compound that has shown high ability for stopping the synthesis of DNA and also acting as a photosensitizer. Nowadays, the radiation dose assessment resource (RADAR) method is known as the most common method for absorbed dose calculation. ^{177}Lu was produced by (n, gamma) reaction in a research reactor. ^{177}Lu-PL_3 was prepared in the optimized condition. The radiochemical yield was checked by ITLC method. The biodistribution of the complex was investigated by intravenously injection to wild-type rats via their tail veins. In this study, the absorbed dose of ^{177}Lu-PL_3 to human organs was estimated by RADAR method. ^{177}Lu was prepared with a specific activity of 2.6-3 GBq.mg^{-1} and radionuclide purity of 99.98 %. Final preparation of the radiolabelled complex showed high radiochemical purity of > 99%. The results show that liver and spleen have received the highest absorbed dose of 1.051 and 0.441 mSv/MBq, respectively. The absorbed dose values for these two dose-limiting tissues suggest more biological studies special in tumor-bearing animals.

***Keywords*—**Internal dosimetry, Lutetium-177, radar.

I. Introduction

NOWADAYS, numerous antitumor agents have been introduced that capable of intercalative binding to DNA [1], and an intercalating moiety is a structural feature of many naturally occurring, clinically useful drugs such as dactinomycin, adriamycin, ellipticine, bleomycin, and their analogues [2]. This observation can be exploited in the design of new antitumor agents. The anticancer activity of lanthanum has, however, been distinctly enhanced by complexation with diverse ligands including chrysin [3], 1-aminocycloalkancarboxylic acid [4] and phenanthroline derivatives [5], [6]. [Tris(1,10-phenanthroline)lanthanum(III)] trithiocyanate (PL_3) is a new compound, in which lanthanum centers a complex built by three 1,10-phenathroline molecules. The rationale behind the synthesis of this compound is that besides lanthanum also the rigid planar 1,10-phenanthroline molecule has been demonstrated to exert distinct effects at least on *in vitro* cultured cells. PL_3 has shown to stop DNA synthesis in CCRF-CEM and Ehrlich ascites cells leading to a cell cycle arrest in G0/G1 [7]-[9]. One other important property of the phenanthroline nucleus is its ability to act as a triplet-state photosensitizer especially in complexes with lanthanides [10]. Complexation of several other metal ions including copper, ruthenium and cobalt with PL has also been used to enhance the anticancer activity [5], [10], [11].

Owing to the suitable decay characteristics of lutetium-177 [T1/2 = 6.73 d, Ebmax = 497 keV, Eg= 113 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 and the special characteristics of PL3, recently, 177Lu-PL3 complex has been developed as a possible therapeutic radiopharmaceutical [12].

The amount of energy deposited in any organs by ionizing radiation, absorbed dose, plays an important role in evaluating the risks associated with the administration of radiopharmaceuticals and thus the maximum amount of activity that should be undertaken [13]. In nuclear medicine, the most commonly used method these days for calculation of the internal dose estimates is the radiation dose assessment resource (RADAR) method [14]. In this work, the absorbed dose to each organ of human for ^{177}Lu-PL_3 was evaluated based on biodistribution studies in rats by RADAR method.

II. 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 for 5 days at Tehran Research Reactor (TRR). Whatman No. 1 was obtained from Whatman (Maidstone, UK). Radio-chromatography was performed by using a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) was used for counting distributed activity in the mice 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 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. The male healthy rabbits and rats were purchased from Pasteur Institute, Tehran, Iran.

A. Production and Quality Control of ^{177}LuCl3 Solution

Lutetium-177 was produced by neutron irradiation of 1 mg of natural Lu_2O_3 (99.999% from Aldrich Co. UK) according to the reported procedures [15] in Tehran Research Reactor at a

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thermal neutron flux of 4×10^{13} n.cm^{-2}.s^{-1} for 5 days. 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 ultra-pure water, to produce a stock solution of final volume of 5 ml. The mixture was filtered through a 0.22 µm biological filter and sent for use in the radiolableing step. The radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy as well as HPGe spectroscopy for the detection of various interfering beta and gamma emitting radionuclides. The radiochemical purity of the $^{177}LuCl_3$ was checked by means of 2 solvent systems for ITLC [A: 10mM DTPA pH.5 and B: ammonium acetate 10%:methanol (1:1)].

B. Synthesis and Quality Control of 177Lu-PL3 Complex

The acidic solution (0.2 ml) of $^{177}LuCl_3$ (111 MBq, 3 mCi) was transferred to a 5 ml-borosilicate vial and heated to dryness us-ing a flow of N2 gas at 50–60°C. Fifty microlitres of PL_3 in absolute ethanol (1 mg/ml ≈ 274 nmoles) was added to the activity-con-taining vial, and the mixture was diluted by the addition of normal saline (4.5 ml) followed by vortexing at 25°C for 30–60 min. The active solution was checked for radiochemical purity by ITLC. The final solution was then passed through a 0.22 mm filter and pH was adjusted to 5.5–7.

For measuring radiochemical purity and radiolabelling yield, a 1 µL sample of the ^{177}Lu-PL_3 complex was spotted on a chroma-tography paper (Whatman No. 1) and developed in 10 mM DTPA solution (pH = 5) as the mobile phase.

C. Biodistribution Studies

The biodistribution ^{177}Lu-PL_3 was determined in wild-type rats. For each piece, 100 µl (150µCi) of radioactive solution was injected directly into a normal rat through their caudal vein. The animals (n = 3) were sacrificed by CO2 asphyxiation at selected times after injection (2 to 168 h) and the percentage of injected dose in the tissues (brain, heart, liver, kidney, testis, spleen, lung, stomach, bladder, etc.) was determined by g-ray scintillation.

D. Dosimetric Studies

The absorbed dose of each human organ was calculated by RADAR method based on biodistribution data in wistar rats. The accumulated activity in animals was extrapolated to the accumulated activity in humans by the proposed method of Sparks et al. (1) [16]:

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

where Ã is the accumulated activity in the source organs and can be calculated by (2):

$$\tilde{A} = \int_{t_1}^{\infty} A(t)\, dt \quad (2)$$

It should be noticed 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. The researchers used a linear approximation between the two experimental points of times. 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 ^{166}Ho. 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 [14].

The radiation absorbed dose was calculated by RADAR formulation [14]:

$$D = N \times DF \quad (3)$$

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

$$DF = \frac{k \sum_i n_i E_i \phi_i}{m} \quad (4)$$

DF represents the physical decay characteristics of the radionuclide, the range of the emitted radiations, and the organ size and configuration [17] expressed in mGy/MBq.s. DFs have been taken from the OLINDA/EXM software [18].

III. Results

A. Production and Quality Control of ^{177}Lu

The radionuclide was prepared in a research reactor according to the regular methods in a range of specific activity 2.6-3 GBq/mg for radiolabeling use. After counting the samples on an HPGe detector for 5 min, two major photons (5.4% of 80.68 keV and 0.9% of 1379.94 keV) were observed. Radionuclidic purity was 99.98 % (Fig. 1).

B. Preparation of ^{177}Lu-PL_3 Complex

Labelling yield increased with increasing PL3 reached more than 99% when the ligand amount reached 0.05 mg after 30 minutes. ITLC showed that the complex s majorly prepared in 30 minutes with 99% radiochemical purity; the remaining 1% is possibly attributed to other Lu ionic species which cannot react with PL_3 (Fig. 2).

C. Biodistribution Studies for ^{177}Lu-PL_3 in Rats

The distribution of injected dose in rat organs up to 168 h after injection of ^{177}Lu-PL_3 (60 µCi/100ul) solution was determined. Based on these results, it was concluded that the largest portion of injected activity of ^{177}Lu-PL_3 was extracted from blood circulation. The complex is majorly accumulated in the reticuloendothelial system, while small amounts of activity in blood, kidney, and bone demonstrate the absence of any free cation released from the complex and/or produced as secondary metabolite.

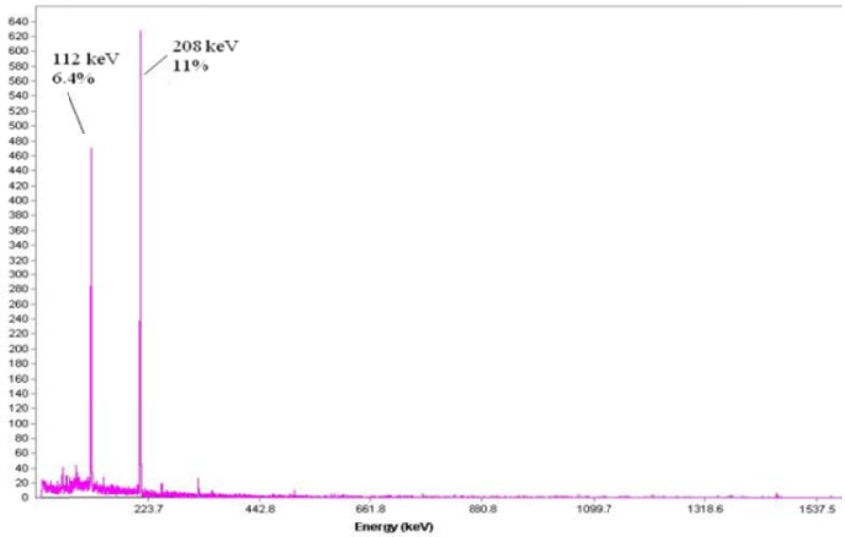

Fig. 1 HPGe spectrum for Lu-177 chloride solution used in this study

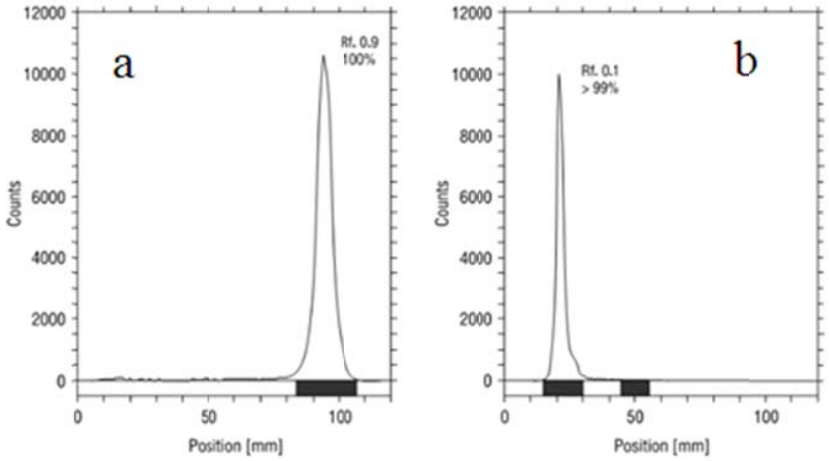

Fig. 2 (a) ITLC chromatograms of $^{177}LuCl_3$ and (b) ITLC chromatograms of ^{177}Lu-PL_3 on Whatman No. 1 paper using DTPA 10 mM solution

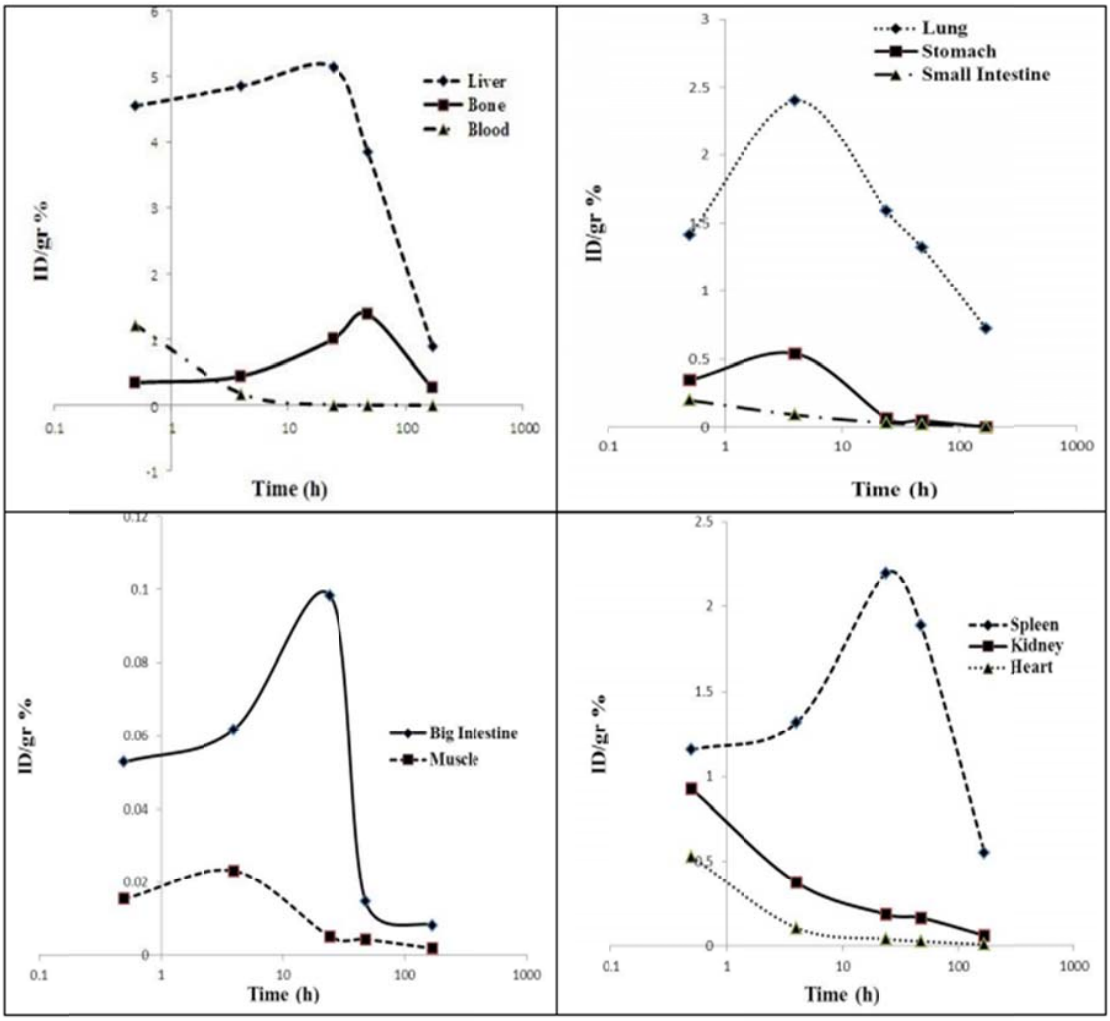

Fig. 3 The clearance curves of ^{177}Lu-PL$_3$ from each organ of the rats

TABLE I
THE ABSORBED DOSE IN EACH HUMAN ORGAN AFTER ^{177}LU-PL $_3$ INJECTION

Tissue	Absorbed dose (mGy/MBq)	Tissue	Absorbed dose (mGy/MBq)
Adrenals	0.016	Muscle	0.007
Brain	0.006	Testes	0.002
Breasts	0.004	Pancreas	0.013
Gallbladder wall	0.021	Red marrow	0.393
LII wall	0.025	Bone volume	0.223
Small intestine	0.005	Ovaries	0.004
Stomach	0.013	Spleen	0.441
ULI wall	0.007	Thymus	0.004
Heart wall	0.022	Thyroid	0.004
Kidneys	0.071	Urinary bladder wall	0.002
Liver	1.051	Uterus	0.003
Lungs	0.261	Total body	0.110

D.Dosimetric Studies

Dosimetric evaluation in human organs was performed by RADAR method based on biodistribution data in rat organs. The clearance curves from each organ of the rats are shown in Fig. 3. The Absorbed dose in each human organ after injection of ^{177}Lu-PL$_3$ is presented in Table I. The highest absorbed dose for this complex is observed in liver with 1.051 mSv/MBq.

IV. CONCLUSION

The ^{177}Lu-PL$_3$ complex can prepare with high radiochemical yield (> 99 %) at optimized conditions; 0.05 mg of PL$_3$ in the presence of 3 mCi Lu^{3+} chloride for 30–60 minutes. IV injection of ^{177}Lu-PL$_3$ complex to male wild-type rats demonstrated activity distribution among rat tissues using sacrification showed different accumulation from free Lu cation. Most of the ^{177}Lu-PL$_3$ was accumulated in the reticuloendothelial system. As shown in Table I, the highest absorbed dose for this complex is observed in liver with 1.051 mSv/MBq. After liver, spleen with 0.441 mSv/MBq has received the highest absorbed dose. Since the liver and spleen are major dose-limiting tissues and absorb the highest dose, therefore further experiments on the accumulation of ^{177}Lu-PL$_3$, specially, in tumor-bearing animals are needed.

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