VIA MEDICA

Nuclear Medicine Review 2010
Vol. 13, No. 2, pp. 49–54
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ISSN 1506–9680

Review

Preparation and quality control of ^{177}Lu-[tris(1,10-phenanthroline) lutetium(III)] complex for therapy

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[Received 17 II 2011; Accepted 18 II 2011]

Abstract

The ^{177}Lu-[*tris*(1,10-phenanthroline)lutetium(III)] complex (^{177}Lu-PQ3) was prepared successfully with high radiochemical purity (> 99%). Lu-177 chloride was obtained by thermal neutron flux (4×10^{13} n.cm^{-2}.s^{-1}) of natural $Lu_2(NO_3)_3$ sample, dissolved in acidic media. The radiochemical yield was checked by measuring the radiochemical purity of the ^{177}Lu-PQ complex by ITLC (10 mM DTPA, pH = 5, as mobile phase). The final complex solution was injected intravenously into wild-type male rats and bio-distribution of the complex was checked for up to 48 hours. The dose limiting organs were shown to be the reticulu-endothelial system. The bio-distribution of the labelled compounds in tumour-bearing animals is under investigation.
Key words: lutetium-177, 1,10-phenanthroquinone, biodistribution

Nuclear Med Rev 2010; 13, 2: 49–54

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Introduction

[Tris(1,10-phenanthroline)lanthanum(III)] (La-PQ_3) has been prepared previously and the rigid planar 1,10-phenanthroline (PQ) molecule has demonstrated distinct effects on *in vitro* cultured cells. The complex has also been shown to stop DNA synthesis in CCRF-CEM and Ehrlich ascites cells leading to a cell cycle arrest in G0/G1 [1, 2], based on the metal chelating ability of PQ [3, 4], several metal ions including copper, ruthenium, and cobalt, has been shown to enhance the anticancer activity of PQ [5–7].

On the other hand, several complexes of vanadium with PQ derivatives have been shown to demonstrate an apoptotic effect *in vivo* and *in vitro* [8–10]. Recently, La-PQ_3 demonstrated anticancer activity *via* potent induction of cell cycle arrest and/or apoptosis and has promising *in vivo* anticancer activity against a human colon cancer xenograft, suggesting La-PQ_3 as a new anticancer metal-drug [11].

In continuation of radionuclidic homolog development of anti-proliferative metal complexes [12, 13], we were interested in developing a lanthanide-based labelled compound from an LaPQ3 lead compound. Among radioactive lanthanides used in nuclear medicine, lutetium-177 is an interesting candidate for therapeutic protocols.

Owing to the suitable decay characteristics of lutetium-177 [T1/2 = 6.73 d, Eβmax = 497 keV, Eγ = 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, ^{177}Lu could be considered as a promising radionuclide in the development of Lu-PQ3 complex as a possible therapeutic radiopharmaceutical (Figure 1).

^{177}Lu-radiopharmaceuticals have been used in the therapy of various diseases and malignancies, such as somatostatin receptor positive tumour radiotherapy [14], radioimmunotherapy [15], bone palliation therapy [16], and radiosynovectomy [17, 18].

In this research, ^{177}Lu-PQ_3 complex was prepared and the effects of various production conditions were investigated on its labelling yield. ^{177}Lu-PQ_3 complex solution was injected intravenously into normal rats for biodistribution determination.

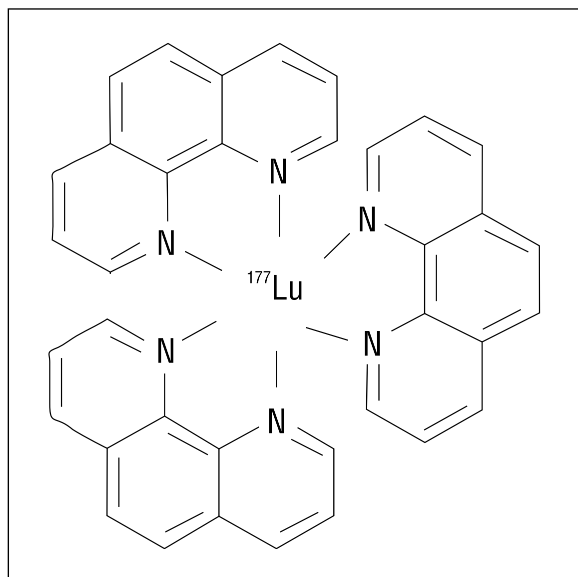

Figure 1. Possible chemical formula for $^{177}Lu\text{-}PQ_3$.

Material and methods

^{177}Lu was produced with a specific activity of approximately 70–80 mCi/mg and radionuclidic purity of 99.98% by irradiation of natural Lu_2O_3 targeted at a thermal neutron flux of approximately 4×10^{13} n/cm^2.s for 5 days at the Tehran Research Reactor (TRR). Whatman No. 1 paper was obtained from Whatman (Maidstone, UK) for instant thin layer chromatography (ITLC). 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 analyser and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in rat organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 208 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. Male healthy rats were purchased from the Pasteur Institute, Tehran, Iran.

Production and quality control of $^{177}LuCl_3$ solution

Lutetium-177 was produced by neutron irradiation of 1 mg of natural $Lu_2(NO_3)_3$ (99.999% from Aldrich Co., UK) according to reported procedures [19] in the Tehran Research Reactor at a thermal neutron flux of 4×10^{13} n/cm^2.s for 5 days. The specific activity of the produced ^{177}Lu was 75 mCi/mg after 5d of irradiation. 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 radiolabelling 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}LuCl3 was checked using 2 solvent systems for ITLC [A: 10mM DTPA pH.5 and B: ammonium acetate 10%:methanol (1:1)].

Synthesis of $^{177}Lu\text{-}PQ_3$ 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 using a flow of N_2 gas at 50–60°C. Fifty microlitres of PQ in absolute ethanol (1 mg/ml ≈ 274 nmoles) was added to the activity-containing 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.

Stability of $^{177}Lu\text{-}PQ_3$ in final formulation

The stability of $^{177}Lu\text{-}PQ_3$ in final preparation was determined by storing the final solution at 25°C for 2 days and performing frequent ITLC analysis to determine radiochemical purity.

Stability of $^{177}Lu\text{-}PQ_3$ in the presence of human serum

The final ^{177}Lu-PQ solution (200 μCi, 50 μl) was incubated in the presence of freshly prepared human serum (300 μl) and kept at 37°C for 2 days. The complex stability was assessed by size exclusion chromatography on a Sepharose column (1 × 30 cm). The column was equilibrated with PBS and eluted at a flow rate of 0.5 ml/min at room temperature; 1 ml fractions were collected and their activities were determined in a dose calibrator; also, the presence of serum proteins in each fraction was determined by Folin-Colciteau® method. The control samples were applied to the column separately, including Lu^{3+} cation, ^{177}Lu-PQ complex, and human serum sample for retention time determination.

Quality control

For measuring radiochemical purity and radiolabelling yield, a 1 μl sample of the $^{177}Lu\text{-}PQ_3$ complex was spotted on a chromatography paper (Whatman No. 1) and developed in 10 mM DTPA solution (pH = 5) as the mobile phase.

Biodistribution studies

The biodistribution of free Lu^{3+} cation as well as $^{177}Lu\text{-}PQ_3$ were determined in wild-type rats. For each piece, 100 μl (150 μCi) of radioactive solution was injected directly into a normal rat through their caudal vein. The animals (n = 3) were sacrificed by CO_2 asphyxiation at selected times after injection (2 to 48 h) and the percentage of injected dose in the tissues (brain, heart, liver, kidney, testis, spleen, lung, stomach, bladder, etc.) was determined by γ-ray scintillation.

Results and discussion

Production and quality control of ^{177}Lu

The radionuclide was prepared in a research reactor according to regular methods, with a range of specific activity 2.5–3 GBq/mg

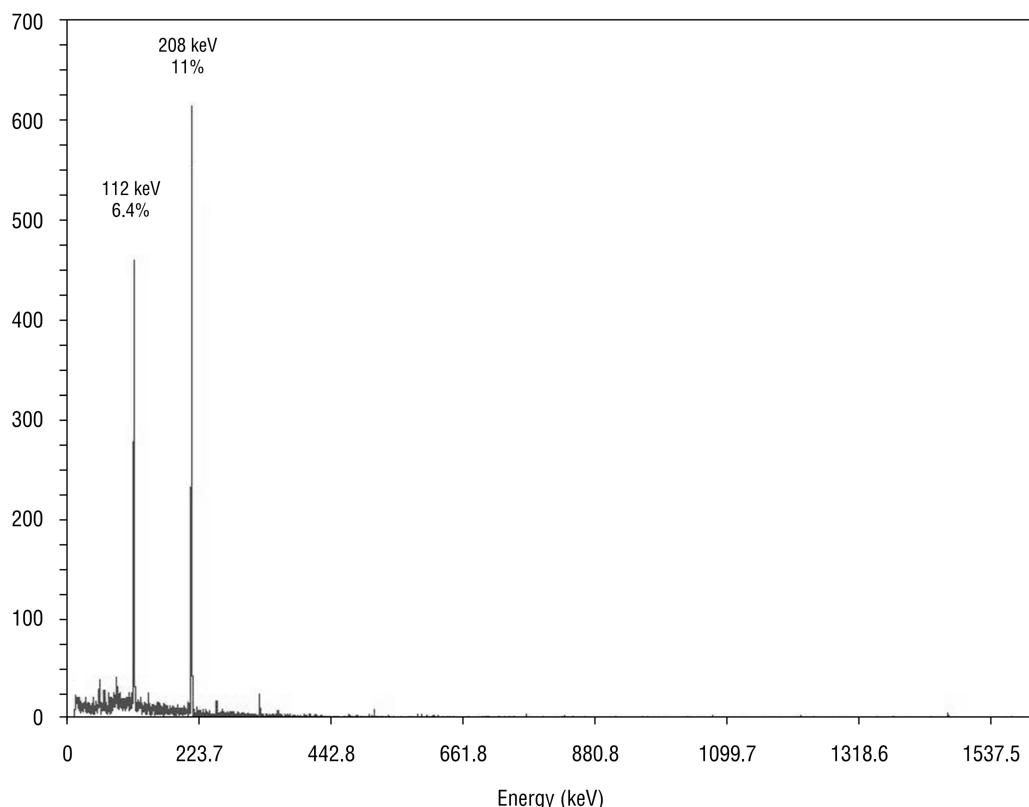

Figure 2. HPGe spectrum for Lu-177 chloride solution used in this study.

for radiolabeling use. After counting the samples on an HPGe detector for 5 hours, two major photons (6.4% of 0.112 MeV and 11% of 0.208 MeV) were observed (Figure 2).

The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated to obtain the desired pH and volume followed by sterile filtering.

The radiochemical purity of the ^{177}Lu solution was checked in two solvent systems; in 10 mM DTPA, the free Lu^{3+} cation is complexed to a more lipophilic LuDTPA form and migrates to a higher R_f, while small radioactive fraction remains at the origin, which could be related to other Lu ionic species not forming LuDTPA complex, such as $LuCl_4^-$, etc. and/or colloids.

On the other hand, ammonium acetate:methanol mixture was also used for the determination of radiochemical purity. The fast eluting species was possibly the ionic Lu-177 cations other than Lu^{3+}, and the remaining fraction at Rf.0 was a possible mixture of Lu^{3+} and/or colloids. Due to the existence of 1% impurity in both cases, the existence of colloids is unlikely (Figure 3).

Preparation of ^{177}Lu-PQ complex

The effect of various factors on the labelling yield of ^{177}Lu-PQ was studied. In higher concentration no significant difference exists on labelling yield for added ^{177}Lu-chloride activity (3 mCi). To investigate the effect of PQ concentration on labelling yield various amounts of the ligand were added to a fixed amount of activity. Labelling yield increased with increasing PQ amount (0.01–0.05 mg) and reached more than 99% when the ligand reached 0.05 mg after 30 minutes. ITLC showed that the complex is majorly prepared in 30 minutes with 99% radiochemical purity; the remaining 1% is possibly attributed to other Lu ionic species which cannot react with PQ (Figure 4).

Stability of ^{177}Lu-PQ complex in final product

The stability of the prepared ^{177}Lu-PQ complex was checked up to 48 hours after preparation. The complex was stable in acidic media (pH = 5.5–7) and its radiochemical purity was above 99% even 48 hours after preparation.

Stability of ^{177}Lu-PQ complex in presence of human serum

A stability test was developed for the complex in the presence of human serum at 37°C. The mixture was then passed through a Sephadex column followed by elution with PBS, and each fraction was checked for radioactivity and the presence of serum proteins by colorimetric method.

Biodistribution studies for ^{177}Lu cation in rats

The animals were sacrificed by CO_2 asphyxiation at selected times after injection (2, 4, 24, and 48 h). Dissection began by drawing blood from the aorta followed by removing heart, spleen, muscle, brain, bone, kidneys, liver, intestine, stomach, lungs, and skin samples. The tissue uptakes were calculated as the percentage of the area under the curve of the related photo peak per gram of tissue (% ID/g) (Figure 5).

The biodistribution of ^{177}Lu cation was determined in wild-type animals for better comparison of 2–48 h post injection. The liver uptake of the cation is comparable to many other

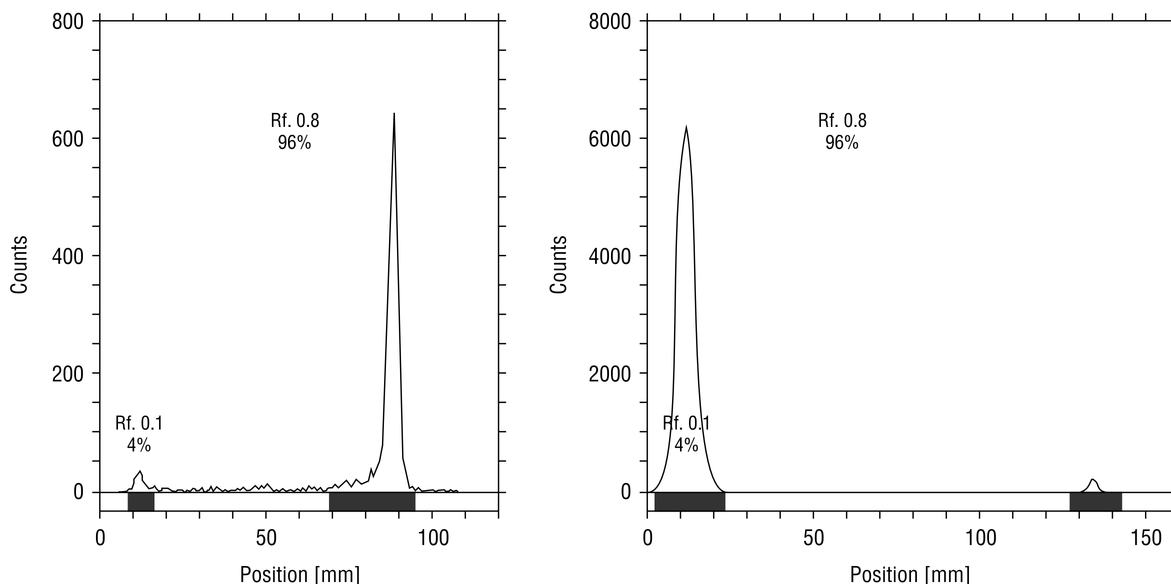

Figure 3. ITLC chromatograms of $^{177}LuCl_3$ solution in DTPA solution (pH = 5) (up) and 10% ammonium acetate:methanol (1:1) solution (down) using Whatman no. 2.

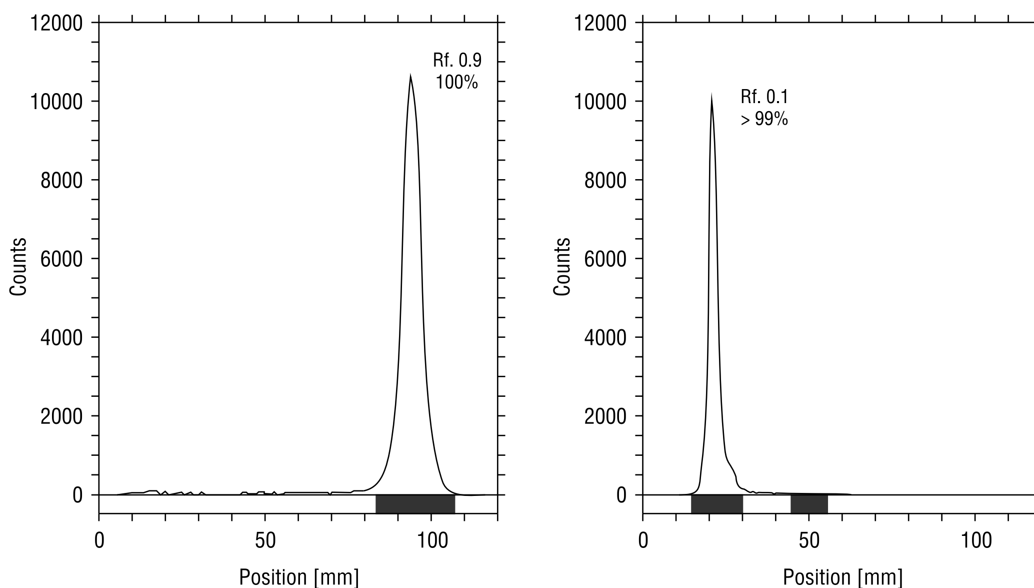

Figure 4. ITLC chromatograms of 177LuCl3 (left) and 177Lu-PQ solution (right) on Whatman no. 2 paper using a methanol: water: acetic acid (4:4:2) mixture.

radio-metals mimicking ferric cation accumulation; about 3 % of the cation accumulates in the liver after 48 h. The transferrin metal uptake and final liver delivery looks to be the possible route of accumulation.

The blood content is low at all time intervals, and this shows the rapid removal of activity in the circulation. Lung, muscle, and skin do not demonstrate significant uptake while it is in accordance with other cation accumulation. A 4% bone uptake is observed for the cation, which remains almost constant after 48 h up to one week (data not shown).

The spleen also has significant uptake, possibly related to reticuloendothelial uptake. As water-soluble cation, Lu^{3+}, kidney plays an important role in excretion *via* urine, especially after 24 h.

Biodistribution studies for $^{177}Lu\text{-}PQ_3$ in rats

The distribution of injected dose in rat organs up to 48 h after injection of $^{177}Lu\text{-}PQ_3$ (60 μCi/100ul) solution was determined. Based on these results, it was concluded that the largest portion of injected activity of $^{177}Lu\text{-}PQ_3$ 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.

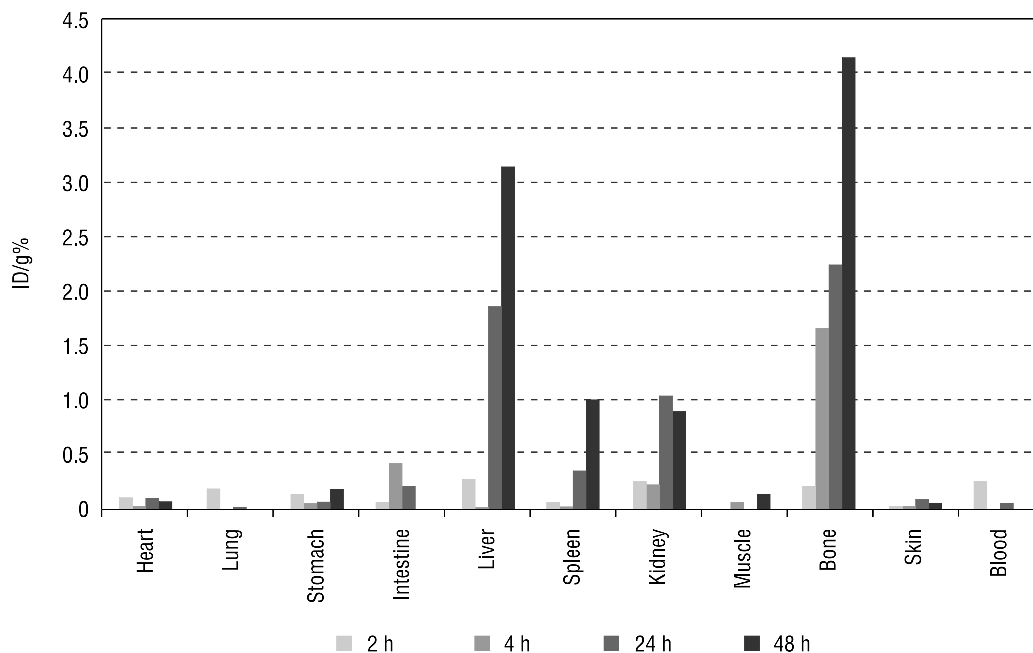

Figure 5. Percentage of injected dose per gram (ID/g %) of $^{177}LuCl_3$ in wild-type rat tissues at 2, 4, 24, and 48 h post injection.

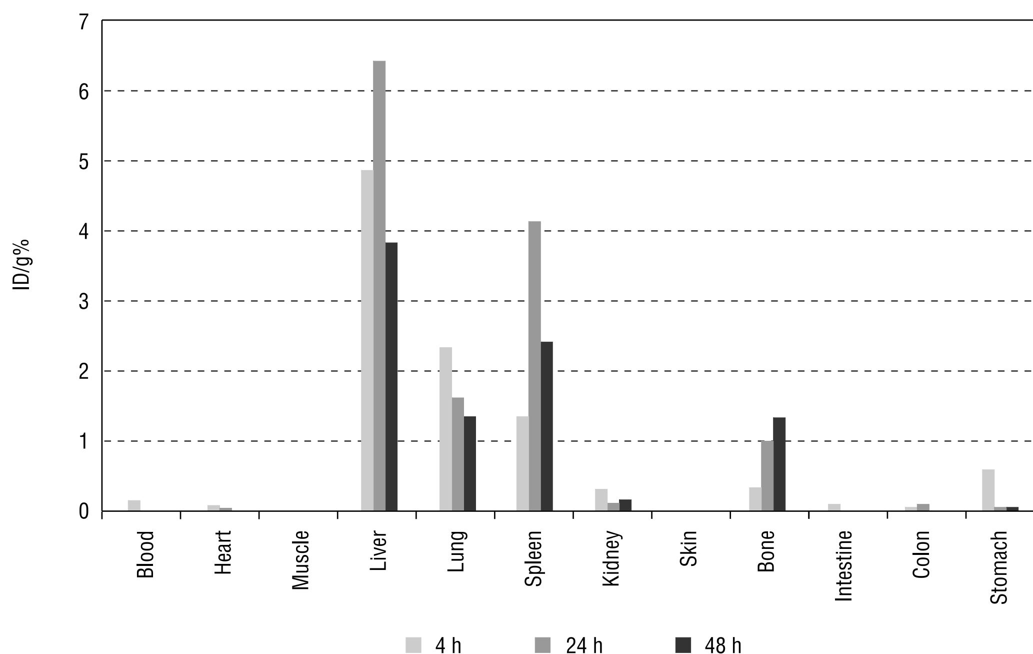

Figure 6. Percentage of injected dose per gram (ID/g %) of $^{177}LuPQ_3$ in wild-type rat tissues at 4, 24, and 48 h post injection.

Conclusions

The ^{177}Lu-PQ complex was prepared with high radiochemical yield (> 99 %) at optimized conditions; 0.05 mg of PQ in the presence of 3 mCi Lu^{3+} chloride for 30–60 minutes. The prepared complex was stable in the final solution at room temperature and the presence of human serum at 37°C and can be used even 24 hours after preparation. IV injection of ^{177}Lu-PQ 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-PQ$_3$ was accumulated in the reticuloendothelial system. Liver and spleen are major dose-limiting tissues. Further experiments on the accumulation of ^{177}Lu-PQ$_3$ in tumour-bearing animals are underway.

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