Original Article

Preparation and biodistribution assessment of low specific activity ^{177}Lu-DOTATOC for optimization studies

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ABSTRACT

Introduction: Somatostatin receptors expressed on a wide range of human tumors, are potential targets for the peptide receptor radionuclide therapy (PRRT). In this study, ^{177}Lu-[DOTA-DPhe^{1}, Tyr^{3}]octreotide (^{177}Lu-DOTATOC) as an agent for PRRT was prepared and its biodistribution was studied in rats.

Methods: The best condition for the preparation of the ^{177}Lu-DOTATOC radiolabeled complex was determined by various experiments. Radiochemical purity of the radiolabeled complex was checked using ITLC method. The stability of the complex in room temperature and in human serum was studied up to 48 h. The biodistribution of ^{177}Lu-DOTATOC solution was investigated in male rats at each selected interval time (2, 4, 24, 48, 72 and 168 h) after injection and compared with the biodistribution of $^{177}LuCl_3$ solution in the same-type rats.

Results: ^{177}Lu-DOTATOC was prepared successfully with radiochemical purity of higher than 99% in 30 min at the optimized conditions. The stability of the radiolabeled complex at room temperature and in human serum at 37 °C showed no decrease in the radiochemical purity even after for 48 h. The biological behavior of the complex showed a major difference uptake with $^{177}LuCl_3$ solution especially in the liver and spleen and also in somatostatin receptor-positive tissues such as pancreas and adrenal.

Conclusion: The results showed that ^{177}Lu-DOTATOC has the comparable pharmacokinetic with the other DOTATOC complexes, while has completely different pattern compared with ^{177}Lu cation.

Key words: DOTATOC; ^{177}Lu; Biodistribution

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INTRODUCTION

Peptide receptor radionuclide therapy (PRRT) utilizing DOTA-conjugated peptides, nowadays, is used for the treatment of various abnormalities with somatostatin receptors. These receptors are expressed on a wide range of human tumors including neuroendocrine, breast, lung, lymphomatic tissue and nervous system and are potential targets for PRRT or peptide-receptor- targeted scintigraphy [1-2].

A series of octreotide analogues were synthesized and used for somatostatin expressing tumor targeting. Octreotide is an eight amino acid synthetic peptide analogue developed to overcome the limitations of native somatostatin such as very low elimination half-time [3]. Among the octreotide analogues, [DOTA-DPhe^1, Tyr^3] octreotide (DOTATOC) indicated advantageous properties in tumor models.

Recently, DOTATOC has been radiolabeled with different radionuclides for diagnostic and therapeutic purposes. While ^{68}Ga-DOTATOC is being used in several European centers in a variety of human tumors [4], various radiolabeled complex of DOTATOC with different radionuclides including gamma and beta emitters such as ^{111}In, ^{177}Lu and ^{90}Y have been developed showing promising results in the diagnosis and treatment of patients with neuroendocrine tumors [5-7].

^{177}Lu is a beta emitter radionuclide with the maximum energy of 497 keV and half-life of 6.73 d. It also emits low-energy γ-rays at 208 and 113 keV with low abundance which permits scintigraphy and subsequent dosimetry during the treatment. Due to these desirable characteristics, several studies have been performed on ^{177}Lu-based PRRT utilizing different somatostatin analogues, such as DOTATOC, [DOTA^0,Tyr^3]octreotate (DOTATATE) and [DOTA^0-1-Nal^3]octreotide (DOTANOC).

The radiolabeled complexes of DOTATOC with ^{90}Y and ^{177}Lu have recently been introduced as valuable new therapeutic options in the treatment of somatostatin receptor-expressing neuroendocrine tumors [6, 8]. Recent studies have demonstrated the improved overall and median survival in the combination of ^{90}Y-DOTATOC and ^{177}Lu-DOTATOC than the use of ^{90}Y-DOTATOC alone [5, 9]. The results of treatment study with ^{90}Y- and ^{177}Lu-DOTATOC on twenty patients with metastatic NETs showed moderate toxicity after treatment with ^{90}Y-DOTATOC in 40% of patients, while no toxicity has been reported for ^{177}Lu-DOTATOC [6].

In the other clinical study with ^{177}Lu-DOTATOC in patients with relapsed NETs after ^{90}Y-DOTATOC treatment, significantly increasing in the creatinine levels and dropping in the mean hemoglobin level after ^{90}Y-DOTATOC therapy has been observed, whereas ^{177}Lu-DOTATOC therapy has been well tolerated by the patients [7]. The recent work by Romer et al. over 910 and 141 patients underwent radiotherapy with ^{90}Y-DOTATOC and ^{177}Lu-DOTATOC, respectively, indicated the higher median survival and lower rate of severe transient haematotoxicities after ^{177}Lu-DOTATOC treatment [10].

Due to the desirable characteristics of ^{177}Lu-DOTATOC, in this study, the authors tried to prepare and optimize its labeling conditions. Besides, biodistribution of the complex was evaluated after intravenous injection into male rats.

METHODS

^{177}Lu was produced by irradiation of enriched Lu_2O_3 target at Tehran Research Reactor (TRR) and using ^{176}Lu (n, gamma) ^{177}Lu nuclear reaction. Whatman No. 3 paper was obtained from Whatman (Maidstone, UK). Radio-chromatography was performed by using a Bioscan AR-2000 radio TLC scanner instrument (Bioscan, France). 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 rat organs. Calculations were based on the 112 keV peak for ^{177}Lu. All values were expressed as mean ± standard deviation and the data were 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 edn. The 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 enriched Lu_2O_3 (^{176}Lu, 52% from ISOTEC Inc.) 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 ultra-pure water. The radionuclidic purity of the solution was checked by means of an HPGe spectrometer for the detection of various interfering gamma emitting radionuclides. The radiochemical purity of the solution was evaluated using instant thin layer chromatography (ITLC) method. For this purpose, 5 µL of the solution was spotted on Whatman No. 3 paper and developed in two solvent systems [A: 10 mmol.L^{-1} diethylene triamine pentaacetic acid (DTPA) at pH.5 and B: 10% ammonium acetate:methanol (1:1)].

Radiolabeling of DOTATOC with $^{177}LuCl_3$

In order to obtain maximum labelling yield, several experiments were carried out by the variation of different reaction parameters. By varying pH (2-7) and temperature (65-100 °C), the effect of these parameters was investigated based on ITLC results.

A stock solution of DOTATOC was prepared by the solution of 115 µg of the ligand in the diluted water. A certain volume of the DOTATOC solution was added to the vial containing 259 MBq of $^{177}LuCl_3$. The pH of the mixture was adjusted using 0.4 M sodium acetate buffer. Then, the mixture was putted in 90 °C water bath for 30 min, while the radiochemical purity of the mixture at the exact interval time was checked by the ITLC method (10, 20 and 30 minutes after preparation). After the addition of 8 mL water to the reaction vial, the mixture was passed through a C_{18} Sep-Pak column which preconditioned with 5 mL ethanol, 10 mL water and 10 mL air, respectively. The column was then washed with 0.5 mL ethanol and 1 mL of 0.9% NaCl.

Quality control of the radiolabeled complex

Radiochemical purity of the radiolabeled complex was checked using ITLC method. Paper chromatography was performed utilizing Whatman No. 3 paper and 0.9 % NaCl as the mobile phase.

Stability studies

The stability of the complex in room temperature and in human serum was studied according to the conventional ITLC method. The radiolabeled complex was kept at room temperature for 48 h while being checked by ITLC at the specified time intervals. For serum stability studies, 37 MBq of ^{177}Lu-DOTATOC was added to 500 µL of freshly prepared human serum and the mixture was incubated at 37 °C for 48 h, aliquots were analyzed by ITLC method.

Biodistribution of $^{177}LuCl_3$ and the radiolabeled complex in rats

The final ^{177}Lu-DOTATOC solution was passed through 0.22 µm biological filter for sterilization and pH was adjusted to 7 by means of 0.9% normal saline. Then, 150 L of the final solution with approximately 3.7 MBq of ^{177}Lu-DOTATOC and $^{177}LuCl_3$ solutions was injected intravenously into male rats through their tail vein. The total amount of radioactivity administered into each animal was measured by counting the 1-mL syringe before and after injection in a dose calibrator with fixed geometry.

The biodistribution of the solutions among tissues were determined by scarification of 3 rats for each selected interval time (2, 4, 24, 48, 72 and 168 h) after injection using the animal care protocols. Blood samples were rapidly taken after scarification. The tissues (heart, kidneys, spleen, stomach, intestine, lung, liver, skin, bladder, bone, muscle, thyroid, adrenal, salivary gland and pancreas) were weighed and rinsed with normal saline and their activities were determined with a p-type coaxial HPGe detector coupled with a multichannel analyzer.

RESULTS

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

^{177}Lu was prepared with the specific activity of 2.6-3 GBq.mg^{-1}. 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 [11]. The radiochemical purity of the ^{177}Lu solution was checked in the 2 solvents systems, indicated the radiochemical purity of higher than 98% [11].

Preparation and quality control of ^{177}Lu-DOTATOC

The best conditions for the preparation of the radiolabeled complex were determined by various experiments. The results indicated the growth of labelling yield with increasing the amount of DOTATOC and reached above 99% by adding 150 µL (105 nmol) of the ligand. It was observed that at the temperature range of 90-100 °C, the maximum complexation yield would be achievable. The effect of pH on the labelling yield was also studied by varying the pH of the reaction mixture from 2 to 7. Maximum yield was observed at pH 4 (Figure 1).

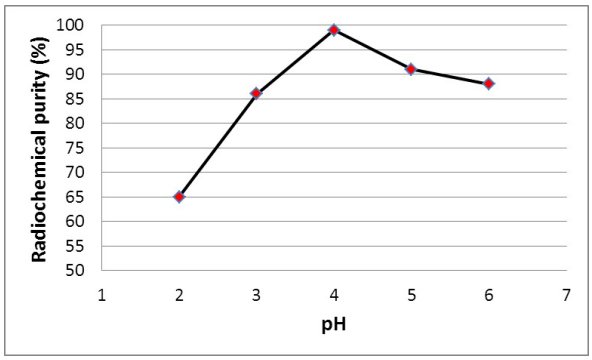

Fig 1. The effect of pH variation on the labelling yield of ^{177}Lu-DOTATOC.

The reaction mixture was incubated at 95 °C temperature for different time periods and 30 min incubation was found to be adequate to yield maximum complexation (Figure 2).

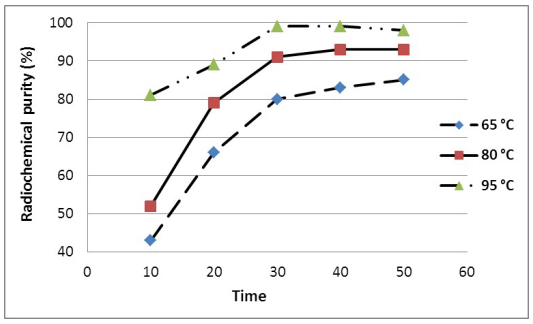

Fig 2. The effect of time and temerature variation on the labelling yielf of ^{177}Lu-DOTATOC.

In the optimized condition, 105 nmol of the ligand was added to the vial containing 259 MBq of $^{177}LuCl_3$ solution and the pH was adjusted to 4. The reaction vial was then heated to 95 ° C for 30 min and then the solution was passed through a C_{18} Sep-Pak column. In this case, thin layer chromatography showed radiochemical purity of higher than 99% (Figure 3).

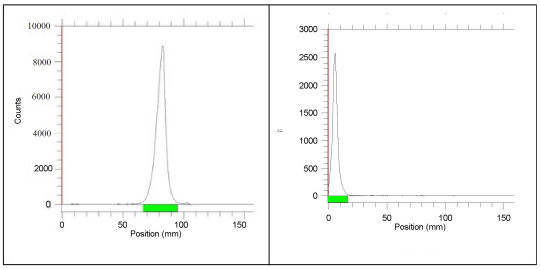

Fig 3. ITLC chromatograms of $^{177}LuCl_3$ (left) and ^{177}Lu-DOTATOC solution (right) on Whatman No. 3 paper using 0.9 % NaCl as the mobile phase.

Stability studies

The stability of the radiolabeled complex at room temperature was checked up to 48 hours. The radiochemical purity of the complex remained more than 98% after 48 h of preparation. Also the stability of the complex in human serum at 37°C showed no decrease in the radiochemical purity even after for 48 h.

Biodistribution of $^{177}LuCl_3$ and the radiolabeled complex in rats

The percentage of injected dose per gram (%ID/g) for different organs after injection of $^{177}LuCl_3$ and ^{177}Lu-DOTATOC was calculated by dividing the activity amount of each tissue (A) to the decay-corrected injected activity and the mass of each organ (Figures 4 and 5).

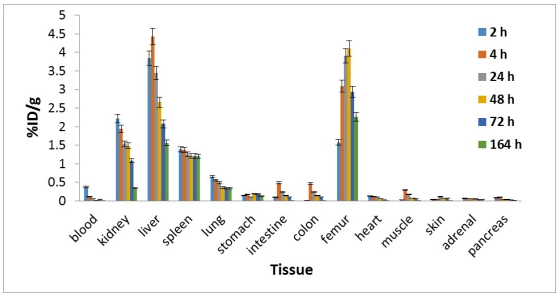

Fig 4. Percentage of injected dose per gram (%ID/g) at 2, 4, 24, 48, 72 and 168 h after intravenously injection of $^{177}LuCl_3$ into rats.

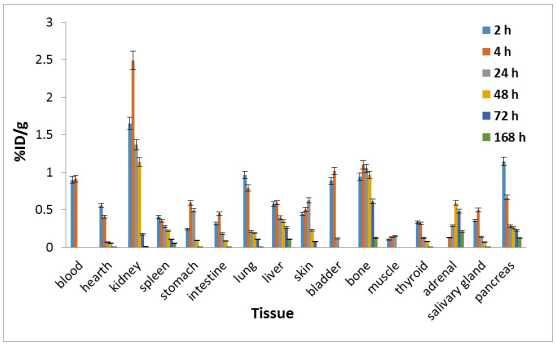

Fig 5. Percentage of injected dose per gram (%ID/g) at 2, 4, 24, 48, 72 and 168 h after intravenously injection of ^{177}Lu-DOTATOC into rats.

DISCUSSION

PRRT is recently suggested as the first- or second-line therapy in inoperable/unresectable NET patients [6]. ^{90}Y and ^{177}Lu are the commonly used radioisotopes for this purpose. While ^{90}Y is the most widely used radionuclide [12], its high-energy emitted β^- particles can penetrate to tissues further away from the target tissue and may lead to the dose-dependent toxicities in patients during and after treatment [13]. In some instances, severe side effects of the kidney and bone marrow may be observed after ^{90}Y- somatostatin labeled therapy [14].

Recently, ^{177}Lu is being considered as a viable alternative for the development of new PRRT agents, especially for smaller metastases [14]. However, the low-energy emitted β^- particles of ^{177}Lu transfers lower target doses, its short emission range results in less irradiation of the non-target tissues. The research

studies have shown the encouraging results of the ^{177}Lu-DOTATOC on tumor regression and also other favorable biological characteristics. After ^{177}Lu-labelled peptides injection to the patients, whole-body imaging should be performed to evaluate the distribution of the radiopharmaceutical and the functional response to PRRT [15].

In this study, ^{177}Lu-DOTATOC was prepared for the first time in the country and the biological behavior of this new complex was investigated after intravenous injection into the rats. The biodistribution results in rats (Figure 3) demonstrated rapid clearance from blood similar to ^{68}Ga-DOTA conjugated peptides [16]. Approximately no activity was found after 2 h in the blood samples. As expected, significant uptake was observed in somatostatin receptor-positive tissues such as pancreas and adrenals which decrease slightly with time.

The kidney with considerable aggregation can be considered as a major body part of excretion. This result is consistent with the other DOTATOC radiolabeled complexes such as ^{90}Y-DOTATOC where the kidney has been introduced as the dose limiting organ [17, 18]. Risk factors for the kidney disease should therefore be identified before treatment and should be considered in the choice of the radiopharmaceutical [8]. ^{177}Lu-labelled peptides with lower energy can be regarded as appropriate options for the preservation of the kidney function. While the maximum kidney uptake was occurred at 4 h post injection of ^{177}Lu-DOTATOC, accumulation in the kidney was decreased with time which is in accordance with the reported work on the biodistribution study of DOTATOC labelled with ^{67}Ga, ^{111}In and ^{90}Y [19].

No considerable accumulation was perceived in the other organs. Rapid blood clearance and negligible uptake in non-target organs introduced this new complex as an ideal therapeutic agent. Although, the use of ^{177}Lu-DOTATOC may be limited by the amount of somatostatin receptor expressed on tumors and also the maximum tolerated dose to the kidneys and the other vital organs.

For better comparison, the biodistribution of $^{177}LuCl_3$ was also investigated in the rats. As it can be seen in the Figure 4, the data for $^{177}LuCl_3$ show that the liver uptake of the cation is comparable to many other radio-lanthanides. Binding of ^{177}Lu by transferrin and transport to the liver appears to be the route of accumulation. The blood content is low at all intervals, which shows the rapid removal of activity in the circulation. The lung, muscle and also skin do not demonstrate significant uptake while it is in accordance with other cations accumulation. A 4.5% bone uptake is observed for the cation at 48 h. The spleen also has uptake (about 1.5%) possibly related to reticuloendothelial uptake. The kidney plays an important role in ^{177}Lu cation excretion (2%).

The accumulation of ^{177}Lu-DOTATOC and $^{177}LuCl_3$ species in main organs is compared in Figure 6.

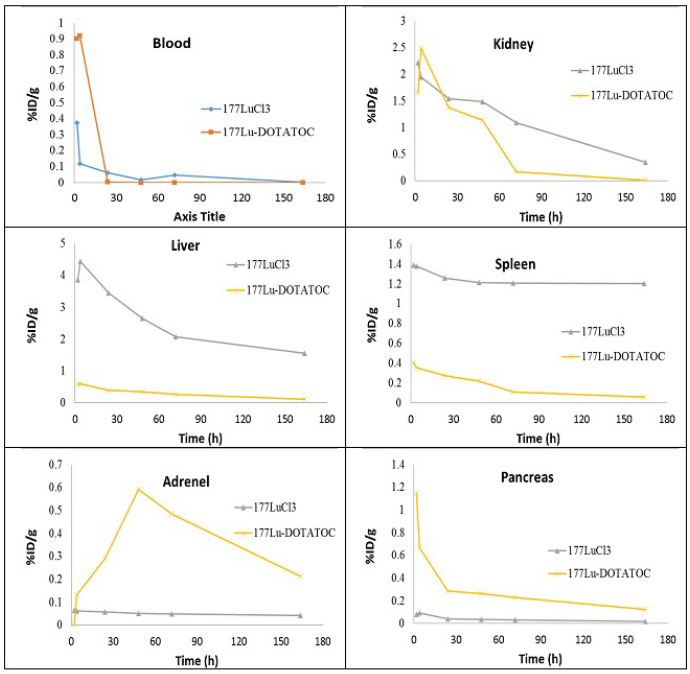

Fig 6. Comparative organ uptake of $^{177}LuCl_3$ and ^{177}Lu-DOTATOC in rats.

As it can be seen in this figure, both compounds are washed out from the circulation after 24 h. Kidney excretion as the main excretion route can be observed for both species that occur due to the water solubility for both of cation and ^{177}Lu-DOTATOC. A major difference in liver uptake is observed for two species. ^{177}Lu-DOTATOC has almost no liver accumulation, which is a major advantage as a therapeutic radiopharmaceutical due to the possibility of increasing the maximum administered dose to the patients. Lu^{3+} cation, being transferred by serum metalloproteins, accumulates in the liver and is excreted through hepatobiliary excretion route, leading to the reduction in liver accumulation. Furthermore, a major difference in spleen uptake is observed for the two species as shown in Figure 6. ^{177}Lu-DOTATOC is not accumulated in spleen which can be again an important benefit as a therapeutic radiopharmaceutical due to the possibility of increasing the maximum administered dose, while Lu-177 cation is present in spleen 2 h post-injection while is almost constant in 168 h after injection. Also, the radiolabeled compound has higher accumulation rather than ^{177}Lu cation in pancreas and adrenal as two somatostatin receptor-positive tissues at all times after injection.

CONCLUSION

^{177}Lu-DOTATOC radiolabeled compound was prepared successfully with radiochemical purity of higher than 99% in 30 min at the optimized condition. The stability of the radiolabeled complex at room temperature and in human serum at 37 °C showed almost no decrease in the radiochemical purity even for 48 h. The biological behavior of this new complex was investigated after intravenously injection into the rats and was compared with the biodistribution of Lu cation in the same-type rats showing a major difference uptake especially in the liver and spleen and also in somatostatin receptor-positive tissues such as pancreas and adrenal. Due to these special characteristics, this agent can be considered as a good therapeutic agent for treatment of patients with neuroendocrine tumors in the country; however, further studies in animal tumor models should be performed.

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