Production of ¹⁷⁷Lu and formulation of Ethylene diamine tetramethylene phosphonate (EDTMP) kits as a bone-seeking radiopharmaceutical

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Background: Owing to its favourable decay characteristics ${}^{177}Lu$ [T_{1/2}= 6.71 d, E_β(max)= 497 keV] is an attractive radionuclide for various therapeutic applications. Ethylene diamine tatramethylene phosphonate (EDTMP) is one of the most widely used ligands which form stable complexes with various radionuclides and all the complexes. Materials and Methods: Enriched ¹⁷⁶Lu₂O₃ was dissolved in 0.1 N HCl and evaporated several times and 176LuCl₃ target was irradiated at 2.6×1013 n.Cm ².S⁻¹ thermal neutron flux for 14 days.¹⁷⁷LuCl₃ was dissolved in 1N HCI. EDTMP was dissolved in double distilled water at pH=7.5-8.5 and freeze-dried kits was radiolabeled with 177LuCl₃ Distribution studies were done in healthy mice. Results: The yield of 177Lu was (~220 TBq/g; 6000 Ci/g), the radionuclidic purity was ~99%.The radiolabeling yield of EDTMP kits at 37°C after 30 min and 4 hours was 98±0.5% and after 72 hours was 90±2.1%, the in vitro stability in human serum at 37°C up to 72 hours post radiolabeling was 85±1.8%. The biodistribution studies of 177Lu-EDTMP and 177LuCl3 in normal mice showed skeleton uptake and low soft-tissue concentration. Conclusion: In this study, we produce ~220 TBq/g (6000 Ci/g) of ¹⁷⁷Lu by neutron activation of ¹⁷⁶Lu in the Tehran Research Reactor. Our results showed ¹⁷⁷Lu-EDTMP as a bone-seeking radiopharmaceutical. Due to its suitable nuclear characteristics 177Lu appears to be worthwhile for palliative therapy of bone metastasis. Iran. J. Radiat. Res., 2010; 7 (4): 229-234

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INTRODUCTION

Radionuclide therapy (RNT) employing open sources of radiotherapeutic agents is fast emerging as an important part of nuclear medicine, primarily due to the development of sophisticated molecular carriers. In order to develop effective radiophrmaceuticals for therapy, it is essential to carefully consider the choice of appropriate radionuclides as well as the carrier moiety with suitable pharmacokinetic properties that could result in good invivo localization and desired excretion. The major criteria for the choice of a radionuclide for radiotherapy are suitable decay characteristics, ease of production and amenable chemistry. As regards the decay characteristics, physical half-life of the radionuclide should match with the biological half-life of the radiopharmaceutical. The energy of the particulate emission should be compatible to the volume of lesion to be irradiated and at the same time should result in minimal dose delivery to the tissues surrounding the site of localization ⁽¹⁾ ⁻⁶⁾. The high-energy beta-particle emitter ¹⁷⁷Lu is candidate for use as therapeutic radiopharmaceuticals. It gives a high local dose for radioimmunotherapy, synovectomy and bone-palliation.

The radionuclide ¹⁷⁷Lu disintegrates by beta (β) decay to three excited levels which are depopulated by six gamma transitions with energies from 71 to 312 KeV and, to an important extent, to the ground state of ¹⁷⁷Hf. The energies of β ⁻ particles from ¹⁷⁷Lu being adequetly low, it is expected to have minimum bone marrow suppression on

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accumulation in skeletal lesions while simultaneously delivering the appropriate dose $^{(9-11)}$.

Emissions of adequate energy gamma photons in low abundance are suitable for carrying out simultaneous scintigraphic studies and dosimetric evaluation ⁽¹¹⁾. Several classes of structurally different phosphonate ligands chelated to different radionuclides emitting moderate energy β particles have been extensively studied for their bone uptake characteristics. The affinity of the coordinated phosphonate ligands for calcium in activity growing bones is considered to be the factor responsible for their selective localization into metastatic lesions. Complexation of radiometals with diphosphonate ligands in aqueous medium produces multiple chelate entities which are not desirable from stability consideration and formulation.

Ethylene diamine tetramethylene phosphonate (EDTMP) is one of the most widely used ligands which forms stable complexes with various radionuclides and all the complexes showed high bone uptake in biodistribution studies ⁽¹²⁻¹⁷⁾.

EDTMP has a high affinity to skeleton and osteoblastic bone metastases and many EDTMP chelates posses a considerably high stability.This stimulated application of the ligand as the *in-vivo* carrier of various radionuclides, intended for both therapy and diagnosis of osteoblastic lesions.

The present study intends to produce ¹⁷⁷Lu in Tehran Research Reactor (TRR) and to formulate EDTMP kits, labeling them with ¹⁷⁷Lu, quality control and biodistribution of it in healthy mice.

MATERIALS AND METHODS

Chemicals and radionuclides

All chemicals were obtained from commercial sources and used without further purification. Enriched $176Lu_2O_3$ (74.1%) was purchased of Campro Scientific Company. EDTMP (Ethylene diamine tetramethylene phosphonic acid) was

Kasei. purchased оf Tokyo Lutetium Oxide, Lu_2O_3 (1.25×10⁻³ mmole, 0.5 mg) was dissolved in 10 ml 0.1 N HCl, by gentle warming. The resultant solution was evaporated near drvness and redissolved in 10 ml of 0.1 N HCl several times. The ¹⁷⁶LuCl₃ was dispensed in quartz capsules and the solvent was evaporated, each capsule contains 10 µg of ¹⁷⁶LuCl₃ and was flame sealed under vacuum and cold welded in aluminum can. 177LuCl₃ was produced by thermal neutron bombardment of it via a (n, y) reaction in TRR at a flux of 2.6×10^{13} n.Cm⁻².S⁻¹ for 14 days. Then the can was opened inside a lead-shielded plant and the product was dissolved in 2 ml 1M HCl by gentle warming.

Ethylene diamine tetramethylene phosphonic acid (EDTMP) was dissolved in distilled water (10 mg/ml), after adjustment of the pH to 7.5-8.5, the mixture was dispensed to 10 ml sterile vials, lyophilized and finally sealed under nitrogen atmosphere. The kits containing 15mg of EDTMP was stored in dark at 2-8°C.

Radionuclide purity, quality control and stability of radiolabeled EDTMP

Radioactivity assay was carried out by measuring the activity in an ISMED 1010 Dose Calibrator (Nuklear-Medizintechnik Dresden GmbH, Dresden, Germany). Radionuclide purity was determined by recording γ -ray spectrum of the appropriately diluted solution of the irradiated target using a multi channel analyzer with high HPGe detector (Silena 2000). A ¹⁵²Eu source (Amersham.Inc.) was used for both energy and efficiency calibration.

Radiochemical purity of 177 LuCl₃ was determined by paper chromatography (Whatman 1mm, Normal saline, Rf =1).

The EDTMP freeze-dried kits were labeled with 50 mCi of ¹⁷⁷LuCl₃, the final volume was 2 ml, after 30 minutes incubation at room temperature the labeling was completed. The labeling yield and stability of the radiopharmaceutical were assessed using paper chromatography (Whatman 1 mm, Normal Saline, Rf =0.1).

Biodistribution studies of ¹⁷⁷LuCl₃

Biodistribution studies were done in the normal mice (20-25 g) 0.1 ml of 177 LuCl₃ (100 µCi), pH =5.5 administered to mice via the tail vein. The animals were killed by CO₂ 24, 48, 120, 144, 168 hours postinjection and blood, kidney, liver, spleen, bone, intestine, colon, muscle, lung were taken out. The radioactivity of the blood pool and samples of weighted tissues and the whole body was measured by a gamma counter. The percentage of the dose per gram of tissue was calculated (%ID/g).

Biodistribution studies of ¹⁷⁷Lu-EDTMP

Biodistribution studies for 177 Lu-EDTMP (pH= 7.5-8.5) was done the same as 177 LuCl₃ and the results were calculated as a percentage of the dose per gram of tissue (% ID/g).

In- vitro stability studies

The stability of ¹⁷⁷Lu-EDTMP complex which was diluted in human serum (5 μ g/ml) was studied at pH= 7.5-8.5 at 37°C for a period of 72 hours after preparation. The

radiochemical purity of the complex was assessed at 1, 3, 24, 48, 72 hours by employing paper chromatography using Wathman 1 mm and normal saline as the eluting solvent.

RESULTS

¹⁷⁷LuCl₃ production

¹⁷⁷Lu from enriched The yield of ¹⁷⁶Lu₂O₃ after 14 days irradiation in Tehran Research Reactor (2.6×10¹³ n. Cm⁻².S⁻¹) was ~220 TBq/g (6000 Ci/g). The radionuclidic purity of ¹⁷⁷Lu was ~99% as estimated by analyzing the y-ray spectrum (figure 1), the major y peaks observed were 113,208,250 and 321 keV, all of which correspond to the photopeaks of ¹⁷⁷Lu. The radioactivity due to ¹⁷⁷mLu (414,418 keV) was insignificant. Table 1 shows thetarget-material composition, irradiation conditions, resulting specific activities, and radionuclide impurity.

Kit labeling

The kit was labeled with 50 mCi of 177 LuCl₃ in 2 ml volume and pH=7.5-8.5. The labeling yield after 30 min was 98 ± 0.5



Figure 1. γ-ray spectrum of ¹⁷⁷Lu.

Table 1. Characteristics of the 177Lu produced for formulation of EDTMP-based radiopharmaceuticals.

Radionuclide	Target material	Irradiation conditions	Specific activity	Radionuclidic impurities
¹⁷⁷ Lu	Lu ₂ O ₃ ,isotopic enrichment, % ¹⁷⁶ Lu(74.1%)	Flux 2.6×10 ns ⁻¹ .Cm ⁻² Duration 2 weeks	6000 Ci/g Lu	^{177m} Lu 0.2 %

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%. The ¹⁷⁷Lu-EDTMP preparations stored for 24 hrs showed radiochemical purity 95 ± 1.2 %. The stability of radiolabeled kit in human serum for 72 hours post labeling was acceptable (90±2.1%).

Biodistribution studies of ¹⁷⁷LuCl₃

The biodistribution studies of 177 LuCl₃ in normal mice are shown in figure 2. It demonstrates skeleton uptake and remaining the activity there and low soft-tissue concentration of 177 LuCl₃.

Biodistribution studies of ¹⁷⁷Lu-EDTMP

Figure 3 shows the biodistribution studies of the $^{177}\mathrm{Lu}\text{-}\mathrm{EDTMP}$ in normal mice.

It revealed skeleton uptake, remaining and low soft-tissue concentration of ¹⁷⁷Lu-EDTMP.

In- vitro stability studies

¹⁷⁷Lu-EDTMP exhibited *in vitro* stability (90 \pm 2.1%) at pH=7.5-8.5 when stored at 37° C for 72 hours post preparation. The complex was found to retain its radiochemical purity to the extent of 85 \pm 1.8% 72 hours after labeling (figure 4).

DISCUSSION

The ${}^{176}LuCl_3$ was irradiated at 2.6×10¹³ n.Cm⁻².S⁻¹ neutron flux for 7 and 14 days,



Figure 2. Biodistribution of 177LuCl₃ in normal mice 24, 48, 120, 144, 168 hours post injection.



Figure 3. Biodistribution of ¹⁷⁷Lu-EDTMP in healthy mice at 24, 48, 120, 144, 168 hours post injection.

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Figure 4. In-vitro stability of 177Lu-EDTMP.

and irradiation for 14 days shows better specific activity. Deionized water was used for preparing freeze-dried kit and radiolabeling because of the competition of metal ions with ¹⁷⁷Lu³⁺ in chelating complexation and decreaseing the yield of labeling.

For labeling the EDTMP kit different activities of $^{177}LuCl_3$ (10, 20, 40, 50, 100 mCi) were used, radiolabeling with 50 mCi showed the best radiolabeling yield and stability of all.

The bone uptake of ¹⁷⁷Lu-EDTMP in mice was found to be high and selective as compared with other tissues. Moreover, ¹⁷⁷Lu-EDTMP was retained in bone throughout the 7-day experimental period. The clearance of ¹⁷⁷Lu-EDTMP from soft tissues was rapid compared with its physical half-life. ¹⁷⁷Lu-EDTMP would bind to bone by bridging of ¹⁷⁷Lu to hydroxyapatite by multidentate phosphonate chelate system. EDTMP chelate has at least eight protonation sites and it binds readily with bi - and trivalent metal radioisotopes, such as ¹⁷⁷Lu, ²²⁷Th, ¹⁵⁴Sm, ¹⁸⁶Re, which were thought to have potential for use in treatment of bone metastases. The femur to other tissue uptake ratios of ¹⁷⁷Lu-EDTMP was high. The clearance of ¹⁷⁷Lu-EDTMP from soft tissues was done by kidney. Our results using ¹⁷⁷LuCl₃ also indicated that the %ID/g of ¹⁷⁷Lu retained in the kidney, liver, spleen, and other tissues was low. The difference in biodistribution between ¹⁷⁷Lu-

EDTMP and ¹⁷⁷LuCl₃ was due to differences in the bioavailability of these complexes. ¹⁷⁷LuCl₃ would initially bind to bone according to the chemical absorption of Lu (III) to hydroxyapatite, while ¹⁷⁷Lu-EDTMP would bind to bone by bridging of ¹⁷⁷Lu to by the hydroxyapatite multidentate phosphate chelate system. Therefore, our comparative study of 177Lu-EDTMP and ¹⁷⁷LuCl₃ demonstrated the efficacy of ¹⁷⁷Lu-EDTMP for bone-affinity radiopharmaceuticals ⁽¹⁸⁻²⁰⁾. All of characteristics were made ¹⁷⁷Lu-EDTMP an ideal radiopharmaceutical for pain palliation in bone metastases.

CONCLUSION

¹⁷⁷Lu has got very good potential as a therapeutic radionuclide. The high thermal neutron cross-section of ¹⁷⁶Lu (n, y) ¹⁷⁷Lu reaction facilitates large-scale production. The present study shows 6000Ci/g of ¹⁷⁷Lu activity could be produced by thermal neutron bombardment at a flux of 2.6×10^{13} n.Cm⁻².S⁻¹ for a period of 14 days using enriched ¹⁷⁶Lu₂O₃ (74.1%). Our results showed that freeze-dried kit developed can be used for preparation of ¹⁷⁷Lu-EDTMP reveals skeleton uptake and low soft-tissue concentration. Due to its suitable nuclear characteristics ¹⁷⁷Lu appears to be worthwhile for palliative therapy of bone metastasis.

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