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Production of ^{177}Lu 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 $^{176}Lu_2O_3$ was dissolved in 0.1 N HCl and evaporated several times and $^{176}LuCl_3$ target was irradiated at 2.6×10^{13} n.Cm^{-2}.S^{-1} thermal neutron flux for 14 days.$^{177}LuCl_3$ was dissolved in 1N HCl. EDTMP was dissolved in double distilled water at pH=7.5-8.5 and freeze-dried kits was radiolabeled with $^{177}LuCl_3$. Distribution studies were done in healthy mice. ***Results:*** The yield of ^{177}Lu 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 ^{177}Lu-EDTMP and $^{177}LuCl_3$ in normal mice showed skeleton uptake and low soft-tissue concentration. ***Conclusion:*** In this study, we produce ~220 TBq/g (6000 Ci/g) of ^{177}Lu by neutron activation of ^{176}Lu in the Tehran Research Reactor. Our results showed ^{177}Lu-EDTMP as a bone-seeking radiopharmaceutical. Due to its suitable nuclear characteristics ^{177}Lu appears to be worthwhile for palliative therapy of bone metastasis. **Iran. J. Radiat. Res., 2010; 7 (4): 229-234**

Keywords: *EDTMP, Bone-seeking radiopharmaceuticals, ^{177}Lu.*

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 *in-vivo* 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 ^{177}Lu is candidate for use as therapeutic radiopharmaceuticals. It gives a high local dose for radioimmunotherapy, synovectomy and bone-palliation.

The radionuclide ^{177}Lu 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 ^{177}Hf. The energies of β^- particles from ^{177}Lu 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 ß- 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 ^{177}Lu in Tehran Research Reactor (TRR) and to formulate EDTMP kits, labeling them with ^{177}Lu, 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 $^{176}Lu_2O_3$ (74.1%) was purchased of Campro Scientific Company. EDTMP (Ethylene diamine tetramethylene phosphonic acid) was purchased of Tokyo Kasei. 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 dryness and redissolved in 10 ml of 0.1 N HCl several times. The $^{176}LuCl_3$ was dispensed in quartz capsules and the solvent was evaporated, each capsule contains 10 µg of $^{176}LuCl_3$ and was flame sealed under vacuum and cold welded in aluminum can. $^{177}LuCl_3$ was produced by thermal neutron bombardment of it via a (n, γ) 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 ^{152}Eu source (Amersham.Inc.) was used for both energy and efficiency calibration.

Radiochemical purity of $^{177}LuCl_3$ was determined by paper chromatography (Whatman 1mm, Normal saline, Rf =1).

The EDTMP freeze-dried kits were labeled with 50 mCi of $^{177}LuCl_3$, 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 $^{177}LuCl_3$

Biodistribution studies were done in the normal mice (20-25 g) 0.1 ml of $^{177}LuCl_3$ (100 µCi), pH =5.5 administered to mice via the tail vein. The animals were killed by CO_2 24, 48, 120, 144, 168 hours post-injection 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 ^{177}Lu-EDTMP

Biodistribution studies for ^{177}Lu-EDTMP (pH= 7.5-8.5) was done the same as $^{177}LuCl_3$ and the results were calculated as a percentage of the dose per gram of tissue (% ID/g).

In- vitro stability studies

The stability of ^{177}Lu-EDTMP complex which was diluted in human serum (5 µg/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

$^{177}LuCl_3$ production

The yield of ^{177}Lu from enriched $^{176}Lu_2O_3$ after 14 days irradiation in Tehran Research Reactor (2.6×10^{13} n. $Cm^{-2}.S^{-1}$) was ~220 TBq/g (6000 Ci/g). The radionuclidic purity of ^{177}Lu was ~99% as estimated by analyzing the γ-ray spectrum (figure 1), the major γ peaks observed were 113,208,250 and 321 keV, all of which correspond to the photopeaks of ^{177}Lu. The radioactivity due to ^{177m}Lu (414,418 keV) was insignificant. Table 1 shows the target-material composition, irradiation conditions, resulting specific activities, and radionuclide impurity.

Kit labeling

The kit was labeled with 50 mCi of $^{177}LuCl_3$ in 2 ml volume and pH=7.5-8.5. The labeling yield after 30 min was 98±0.5

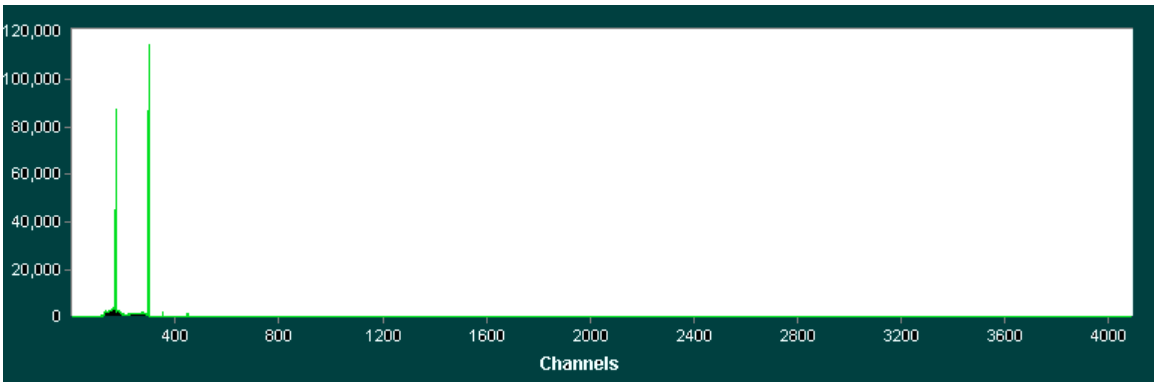

Figure 1. γ-ray spectrum of ^{177}Lu.

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

Radionuclide	Target material	Irradiation conditions	Specific activity	Radionuclidic impurities
^{177}Lu	Lu_2O_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 %

%. The ^{177}Lu-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 $^{177}LuCl_3$

The biodistribution studies of $^{177}LuCl_3$ in normal mice are shown in figure 2. It demonstrates skeleton uptake and remaining the activity there and low soft-tissue concentration of $^{177}LuCl_3$.

Biodistribution studies of ^{177}Lu-EDTMP

Figure 3 shows the biodistribution studies of the ^{177}Lu-EDTMP in normal mice. It revealed skeleton uptake, remaining and low soft-tissue concentration of ^{177}Lu-EDTMP.

In- vitro stability studies

^{177}Lu-EDTMP exhibited *in vitro* stability (90±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±1.8% 72 hours after labeling (figure 4).

DISCUSSION

The $^{176}LuCl_3$ was irradiated at 2.6×10^{13} $n.Cm^{-2}.S^{-1}$ neutron flux for 7 and 14 days,

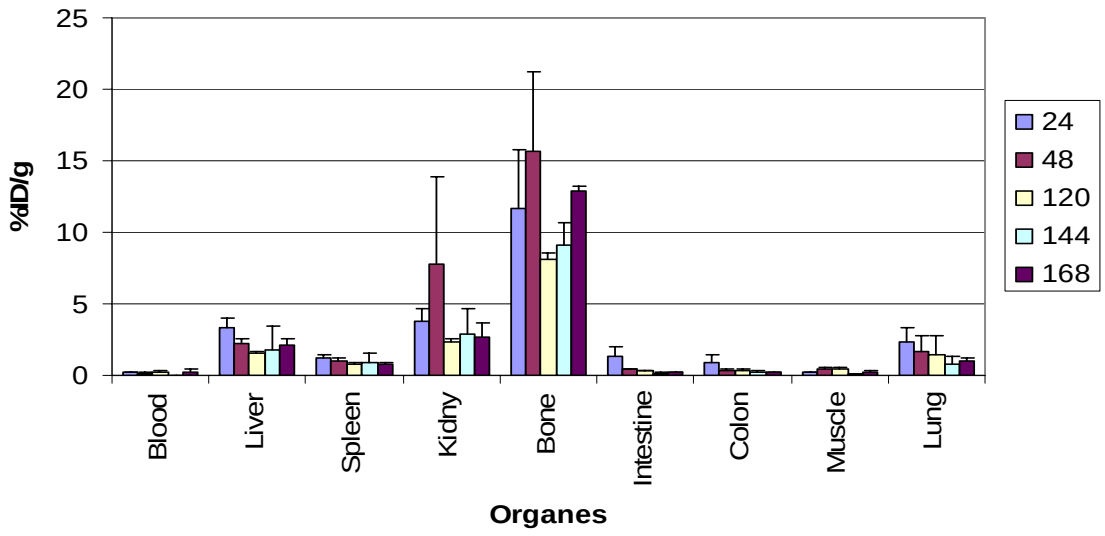

Figure 2. Biodistribution of $^{177}LuCl_3$ in normal mice 24, 48, 120, 144, 168 hours post injection.

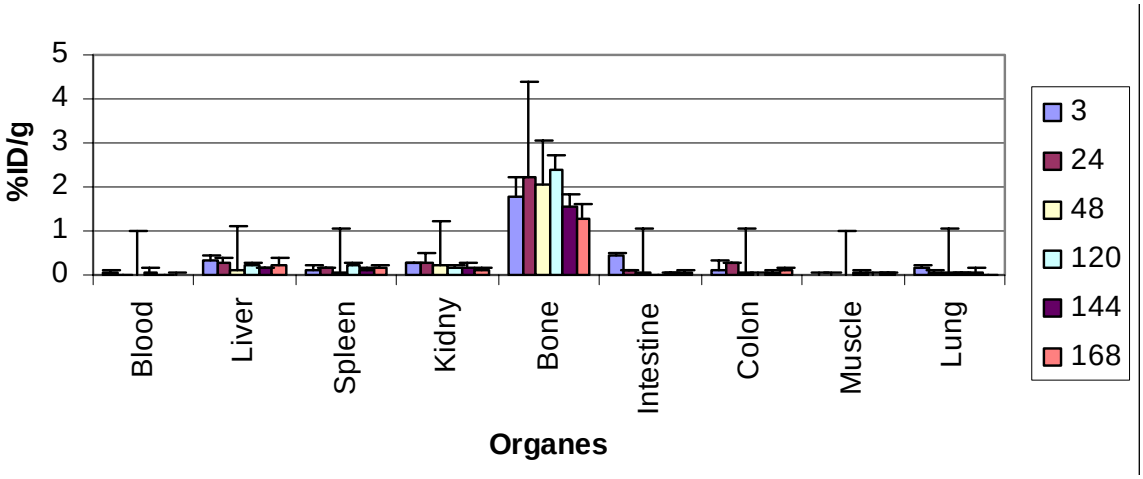

Figure 3. Biodistribution of ^{177}Lu-EDTMP in healthy mice at 24, 48, 120, 144, 168 hours post injection.

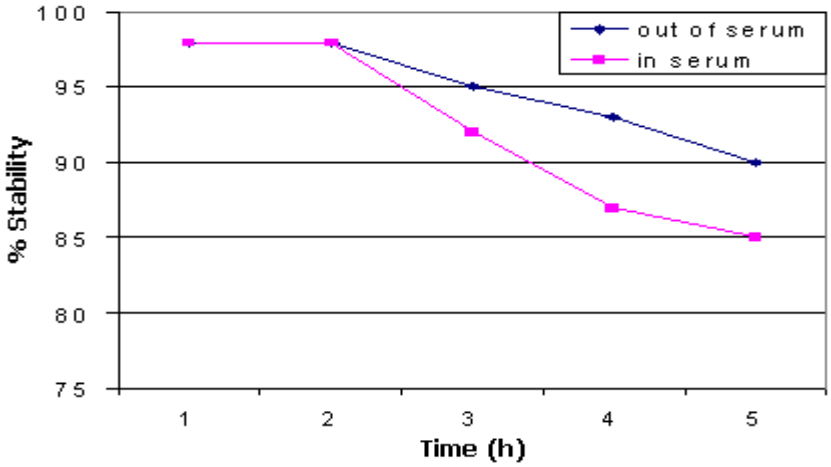

Figure 4. *In-vitro* stability of ^{177}Lu-EDTMP.

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 $^{177}Lu^{3+}$ 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 ^{177}Lu-EDTMP in mice was found to be high and selective as compared with other tissues. Moreover, ^{177}Lu-EDTMP was retained in bone throughout the 7-day experimental period. The clearance of ^{177}Lu-EDTMP from soft tissues was rapid compared with its physical half-life. ^{177}Lu-EDTMP would bind to bone by bridging of ^{177}Lu 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 ^{177}Lu, ^{227}Th, ^{154}Sm, ^{186}Re, which were thought to have potential for use in treatment of bone metastases. The femur to other tissue uptake ratios of ^{177}Lu-EDTMP was high. The clearance of ^{177}Lu-EDTMP from soft tissues was done by kidney. Our results using $^{177}LuCl_3$ also indicated that the %ID/g of ^{177}Lu retained in the kidney, liver, spleen, and other tissues was low. The difference in biodistribution between ^{177}Lu-EDTMP and $^{177}LuCl_3$ was due to differences in the bioavailability of these complexes. $^{177}LuCl_3$ would initially bind to bone according to the chemical absorption of Lu (III) to hydroxyapatite, while ^{177}Lu-EDTMP would bind to bone by bridging of ^{177}Lu to hydroxyapatite by the multidentate phosphate chelate system. Therefore, our comparative study of ^{177}Lu-EDTMP and $^{177}LuCl_3$ demonstrated the efficacy of ^{177}Lu-EDTMP for bone-affinity radiopharmaceuticals [18-20]. All of characteristics were made ^{177}Lu-EDTMP an ideal radiopharmaceutical for pain palliation in bone metastases.

CONCLUSION

^{177}Lu has got very good potential as a therapeutic radionuclide. The high thermal neutron cross-section of ^{176}Lu (n, γ) ^{177}Lu reaction facilitates large-scale production. The present study shows 6000Ci/g of ^{177}Lu 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 $^{176}Lu_2O_3$ (74.1%). Our results showed that freeze-dried kit developed can be used for preparation of ^{177}Lu-EDTMP reveals skeleton uptake and low soft-tissue concentration. Due to its suitable nuclear characteristics ^{177}Lu appears to be worthwhile for palliative therapy of bone metastasis.

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