Original Article

^{175}Yb-TTHMP as a good candidate for bone pain palliation and substitute of other radiopharmaceuticals

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ABSTRACT

Bone metastasis is one of the most frequent causes of pain in cancer patients. Different radioisotopes such as P-32, Sm-153, Ho-166, Lu-177, and Re-186 with several chemical ligands as ethylenediaminetetramethylene phosphonic acid (EDTMP), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylene phosphonic acid (DOTMP), and propylenediaminetetramethylene phosphonate (PDTMP) are recommended for bone pain palliation. In this work, ^{175}Yb-triethylenetetraminehexamethylene phosphonic acid (TTHMP) was produced as a proper alternative to other radiopharmaceuticals. Relatively long half-life ($T_{1/2}$ = 4.18 days), maximum energy beta particle E_{β} = 470 keV (86.5%), low abundance gamma emission 396 keV (6.4%), 286 keV (3.01%), 113.8 keV (1.88%) and low cost are considered advantageous of Yb-175 are to wider usage of this isotope; in addition, TTHMP is an ideal carrier moiety for bone metastases. Production, quality control, and biodistribution studies of ^{175}Yb-TTHMP were targeted. Yb-175 chloride was obtained by thermal neutron bombardment of a natural Yb_2O_3 sample at Tehran Research Reactor (TRR), radiolabeling was completed in 1 h by the addition of TTHMP at the room temperature and pH was 7.5-8, radiochemical purity was higher than 95%. Biodistribution studies in normal rats were carried out. The results showed favorable biodistribution features of ^{175}Yb-TTHMP, indicating significant accumulation in bone tissues compared with clinically used bone-seeking radiopharmaceuticals. This research presents ^{175}Yb-TTHMP can be a good candidate for bone pain palliation and substitute of other radiopharmaceuticals, however, further biological studies in other mammals are still needed.

Keywords: Biodistribution, bone metastases, radiopharmaceutical, TTHMP, Ytterbium-175

INTRODUCTION

Bone metastases are particularly common in patients with breast, prostate, and lung cancer.[1] In 80% of the cases, bone metastases are located in several sites and can produce some complications, such as pathologic fracture, mainly femur and humerus; hypercalcemia (10% of cases); and spinal cord compression (5% of cases).[2] Compared to the other conventional methods, such as use of analgesics and external beam radiotherapy, systematic palliative therapy using suitable bone-seeking radiopharmaceuticals have emerged as the most efficacious treatment modality for patients with multiple skeletal lesions.[3,4] The major challenge in developing effective radiopharmaceuticals for palliative treatment of bone pain due to skeletal metastasis is to deliver adequate dose of ionizing radiation at the skeletal lesion sites with minimum radiation-induced bone marrow suppression.[5] Radioactive isotopes of phosphorus (^{32}P) and strontium (^{89}Sr) were the first radiopharmaceuticals used for treatment of skeletal metastases in patients.[6,7] Other beta-emitting radionuclides such as ^{186}Re, ^{153}Sm, ^{177}Lu, ^{117m}Sn, ^{166}Ho, and ^{175}Yb are recommended for this purpose.[8-14] All agents have benefits and risks. The agents differ in terms of efficacy, duration of pain palliation, tumoricidal effects, ability to repeat treatments, toxicity, and other aspects.[15]

Ytterbium-175 is one of radioisotopes that can be used for bone pain palliation. The thermal neutron cross-section of ^{174}Yb is 63.2 barns.[16] Therefore, it is possible to produce ^{175}Yb in adequate specific activity using medium flux reactors. It decays

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by emission of beta particles 470 keV maximum energy (86.5%) to stable ^{175}Lu. The prominent gamma-rays emitted from ^{175}Yb are 113.8 keV (1.88%), 286 keV (3.01%), and 396 keV (6.4%); these energies are suitable for imaging studies and low dose delivering for the patients.[17] Also, the physical half-life of ^{175}Yb is relatively longer (compared to ^{153}Sm or ^{166}Ho) and provide logistic advantages for facilitating supply to nuclear medicine centers far away from the reactors.

Multidentate polyaminopolyphosphonic acid ligands are known to form stable chelates with many metals including lanthanides. Among them, triethylenetetraminehexamethylene phosphonic acid (TTHMP) can be envisaged as an ideal carrier moiety, for the development of beta emitter-based radiopharmaceuticals, for bone palliation.[18] In this research; preparation, quality control, and biodistribution study of ^{175}Yb-TTHMP complex in animal model has been carried out.

MATERIALS AND METHODS

Natural ytterbium oxide was purchased from Isotec Inc, USA. All chemical components were obtained from Sigma-Aldrich Chemical Co. UK. All radioactivities counting related to paper chromatography were carried out by using a NaI (Tl) scintillation counter on adjustment of the baseline at 396 keV. The activity as well as the radionuclidic purity of the ^{175}Yb produced was ascertained by gamma spectroscopy on the base of 396 keV peak by using the high-purity germanium (HPGe) detector and beta-spectroscopy was carried out by the Wallac 1220 Quantulus liquid scintillation spectrometer. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations. All the values were expressed as mean ± standard deviation (mean ± SD).

Production and quality control of $^{175}YbCl_3$

^{175}Yb was produced by thermal neutron bombardment of natural Yb_2O_3 target with isotopic purity of 31.8% for ^{174}Yb at Tehran Research Reactor (TRR) with neutron flux of 3×10^{13} n/cm^2/s by production scheme; $^{174}Yb(n,\gamma)^{175}Yb \rightarrow {}^{175}Lu$ (stable). A weighed amount of Yb_2O_3 powder was flame sealed into a quartz ampule under vacuum and cold welded into aluminum can. Irradiation was carried out for 7 days. Irradiated Yb_2O_3 powder was dissolved in 1 ml of 0.1 M HCl by gentle warming. The resultant solution was evaporated to near dryness and reconstituted in 1 ml of double-distilled water. For radionuclidic purity determination, the samples were checked by gamma-ray spectroscopy on an HPGe detector. The radiochemical purity of $^{175}YbCl_3$ was checked using Whatman no. 3 chromatography paper (was obtained from Maidstone, UK) in NH_4OH:MeOH:H_2O (1:10:20) system.

Synthesis of TTHMP

The experimental procedure for the synthesis of TTHMP ligand was in accordance with other bisphosphonates as reported. For synthesis of TTHMP, a quantity of 0.48 g (3.3 mmol) of triethylenetetramine was dissolved in 0.75 mL of concentrated HCl and a concentrated aqueous solution of 1.62 g (20 mmol) of phosphorous acid. The resulting solution was heated to reflux temperature and 3.2 mL of 37% aqueous formaldehyde solution (40 mmol) was added dropwise in the course of 1 h to the refluxing solution and refluxing was continued for another 1 h. Chemical structure of TTHMP is shown in Figure 1.

Radiolabeling of TTHMP with $^{175}YbCl_3$

$^{175}YbCl_3$ (2.85 mCi) dissolved in 0.1 mL of acidic medium (0.1 M HCl) was transferred to a 2 mL vial and the mixture was evaporated by slight warming under a nitrogen flow. Volumes of TTHMP solution (10 mg/mL) were added to activity containing vials. The mixtures were stirred at room temperature for up to 30-60 min. The active solution was checked for radiochemical purity three times for every 2 h by instant thin layer chromatography (ITLC). The pH of final solution was 7.5-8 and then filtered through a 0.22 μm millipore filter for sterilization.

Stability of ^{175}Yb-TTHMP complex

Frequent analyses have been performed using Whatman no. 3 chromatography paper in NH_4OH:MeOH:H_2O (1:10:20) system to determine the stability of the ^{175}Yb-TTHMP.

Serum stability studies

The stability of ^{175}Yb-TTHMP solution (200 μCi, 50 μl) was checked in presence of freshly prepared human serum (150 μl) at 37°C and was frequently tested for 2 days using above mentioned chromatography system.

Biodistribution studies in rats

In order to investigate biodistribution of ^{175}Yb-TTHMP, the data for biodistribution of free ytterbium cation in animals should be obtained. Biodistribution studies of ^{175}Yb cation and ^{175}Yb-TTHMP were carried out in rats weighing 200-250 g with three rats for each time point. The prepared formulation (150-200 μl, 100-150 μCi) were injected through the tail vein of the rats. The rats were sacrificed post-anesthesia at 2, 4, and 48 h and 4 and 8 days post injection. The tissues and organs were excised and the activity associated with each organ was measured in a NaI (Tl) scintillation counter. For each time interval three rats were used. Distribution of the activity in different organs was calculated as percentage of injected activity per gram of organ (ID/g%).

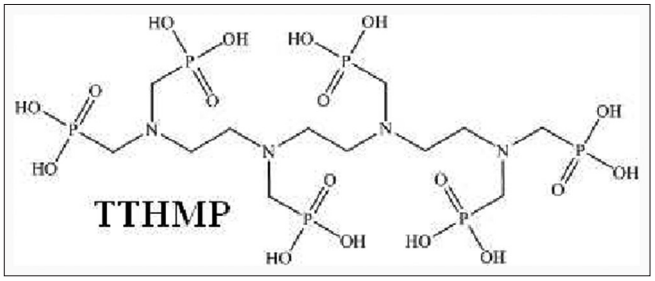

Figure 1: Chemical structure of ligand

RESULTS AND DISCUSSION

Production of ^{175}Yb

Around 1.3-1.5 GBq/g (35-40 mCi/mg) of ^{175}Yb activity was obtained from natural Yb_2O_3 target neutron bombarding. Irradiation of natural Yb_2O_3 target also results in the formation of ^{169}Yb and ^{177}Lu as radionuclidic impurities. A typical gamma-ray spectrum of irradiated ytterbium is shown in Figure 2. The observed photo peaks correspond to the photo peaks of ^{175}Yb (113, 286, and 396 keV), ^{169}Yb (63, 110, 130, 177,198, 261, and 307 keV), and ^{177}Lu (208 and 112 keV).

Labeling optimization studies

In order to obtain the highest labeling yield, a quantitative study was performed using amount of $^{175}YbCl_3$ with different molar ratio of TTHMP at room temperature (25°C). The results are shown in Table 1.

Stability of ^{175}Yb-TTHMP in final formulation

The stability of the ^{175}Yb-TTHMP complex prepared under optimized reaction conditions was studied and it was observed that the complex has excellent stability at room temperature. The complex remained stable to the extent of 98% up to 8 days. The $^{175}Yb^{3+}$ remained at the origin (R_f = 0.0-0.1) and the ^{175}Yb-TTHMP complex migrates with the solvent to higher Rf (R_f = 0.8).

Biodistribution studies

The tissue distribution of $^{175}YbCl_3$ and ^{175}Yb-TTHMP determined in rats over 8 days is shown in Figures 3 and 4, respectively. Specific activity of different organs was calculated as the percentage of injected dose per gram using NaI (Tl) detector. It can be seen from Figure 3 accumulation of free Yb^{3+} cation in the vital organs, that is, kidney, liver, lung, spleen, and heart are appreciable. Figure 4 demonstrates favorable features of ^{175}Yb-TTHMP; such as, significant bone uptake-retention and rapid blood clearance.

For the better comparison of two sample's behavior, vital organ uptake for free Yb-175 and ^{175}Yb-TTHMP are described. Figure 5 demonstrates the blood accumulation from 2 h to 8 days. Because of the intravenous injection of solutions, accumulation of activities in blood is in the highest value at first 2 h and then $^{175}YbCl_3$ and ^{175}Yb-TTHMP are washed out from the circulation after 2 h, although the mechanisms of washout from blood are different.

Figure 6 demonstrates the bone accumulation from 2 h to 8 days. ^{175}Yb-TTHMP complex was rapidly taken up in the bone in 4 h after injection (ID/g% = 2.29) and remained almost constant up to 8 days (ID/g% = 2.85). Instead, uptake of the free Yb-175 increased slightly, but never exceeded 2%.

In the case of the kidney, ^{175}Yb-TTHMP is rapidly taken up in bone and the trapping continues and almost no blood circulation is observed. The concentration of ^{175}Yb-TTHMP in kidney decreased from (ID/g% = 0.18) in 2 h to (ID/g% = 0.12) in 4 h and after 8 days it was negligible. But free ^{175}Yb as a water-soluble cation is washed out through kidney in 8 days [Figure 7].

Yb^{3+} cation being transferred by serum metalloproteinase accumulates in liver and is excreted through hepatobiliary

Table 1: The effect of various molar ratios of Yb:TTHMP

Yb:TTHMP	Radiochemical yield
1:1	37.8±0.40
1:2.5	57.4±0.20
1:10	88.5±0.18
1:25	95.1±0.19
1:30	91.2±0.22

TTHMP: Triethylenetetraminehexamethylene phosphonic acid

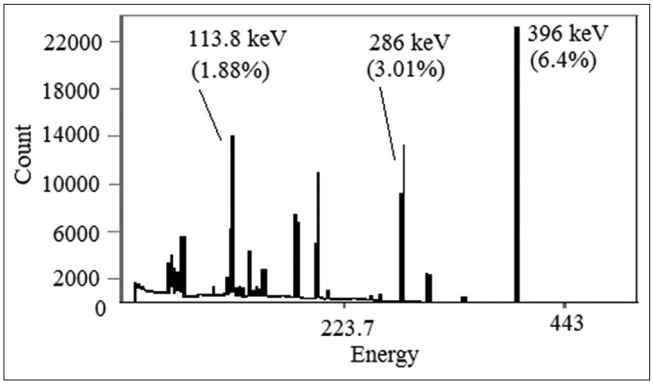

Figure 2: The high-purity germanium (HPGe) spectrum for Yb-175

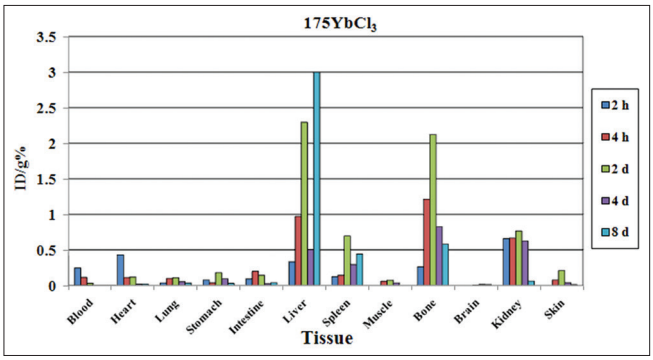

Figure 3: Percentage of injected dose per gram of $^{175}YbCl_3$ in rats

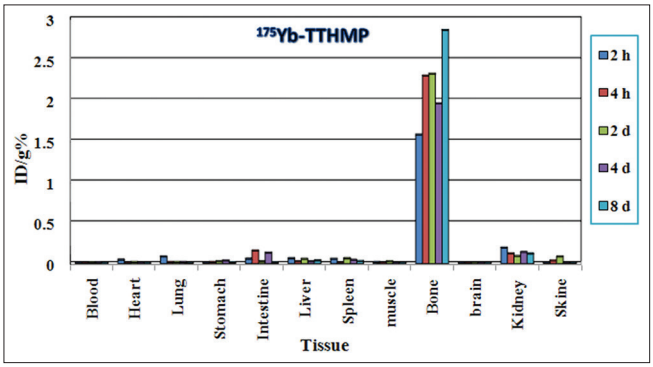

Figure 4: Percentage of injected dose per gram of ^{175}Yb-triethylenetetramine hexamethylene phosphonic acid (TTHMP) in rats

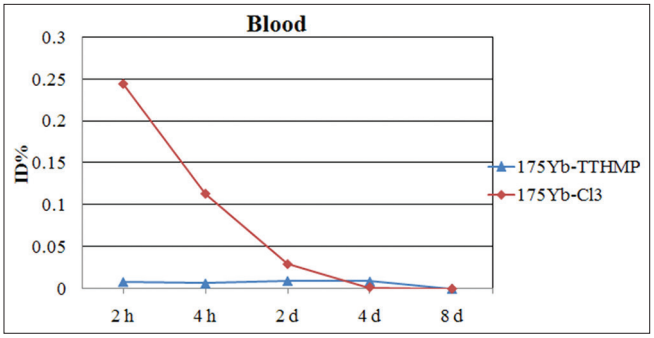

Figure 5: Comparative blood activity for ^{175}Yb-TTHMP and $^{175}YbCl_3$ in rats

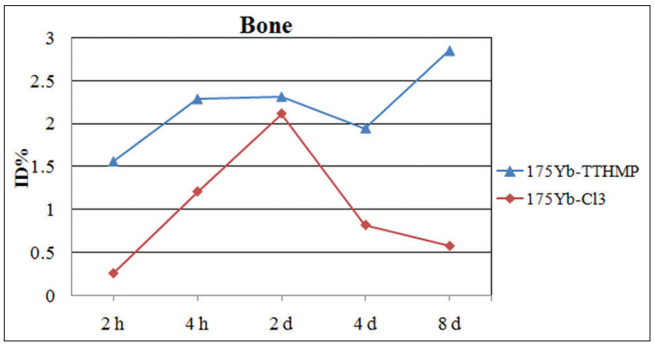

Figure 6: Comparative bone activity for ^{175}Yb-TTHMP and $^{175}YbCl_3$ in rats

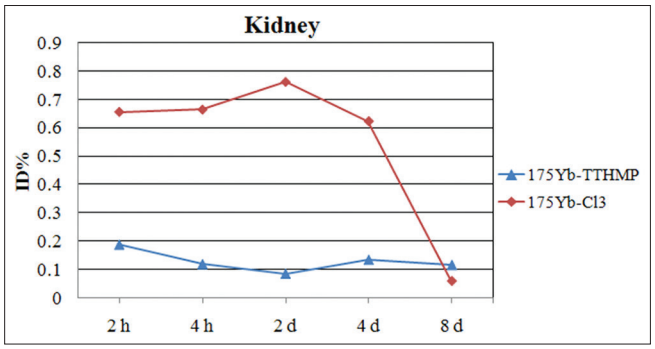

Figure 7: Comparative kidney activity for ^{175}Yb-TTHMP and $^{175}YbCl_3$ in rats

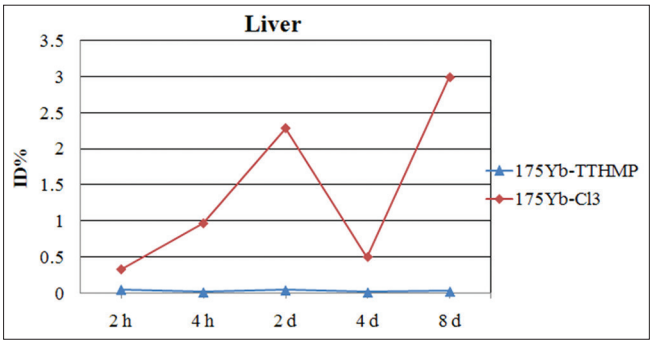

Figure 8: Comparative liver activity for ^{175}Yb-TTHMP and $^{175}YbCl_3$ in rats

excretion route, leading to reduction in liver accumulation, while ^{175}Yb-TTHMP has almost no liver accumulation. This is a major advantage as a therapeutic radiopharmaceutical due to the possibility of increasing the maximum inject able dose [Figure 8].

CONCLUSION

From the animal tests and quality control data results, ^{175}Yb-TTHMP shows suitable features to be used as alternative radiopharmaceutical for bone pain palliation. It was prepared and quality control was carried out in optimized conditions. Labeling and quality control took 1 h, radiochemical purity was higher than 95%, and radionuclidic purity was acceptable. The radiolabeled complex was stable in human serum for least 2 days. The biodistribution data on normal rats showed at least 70% accumulation of ^{175}Yb-TTHMP is in the bone tissues. Although it is not available in high specific activities, the uni-elemental abundance makes it an accessible and inexpensive radionuclide; and the obtained specific activity is enough for radiolabeling of small molecules at radiopharmaceutical grades. Therefore, in this situation and considering all of the excellent features of ^{175}Yb-TTHMP, this radiopharmaceutical can be used for effective management of bone pain palliation of skeletal metastases.

Animal rights

The institutional and international guide for the care and use of laboratory animals was followed. See the experimental part for details.

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