Iranian Journal of Pharmaceutical Sciences
Spring 2012: 8(2): 135-141
ijps.sums.ac.ir

Original Article

Production, Radiolabeling and Biodistribution Studies of ^{175}Yb-DOTMP as Bone Pain Palliation

Laleh Safarzadeh[a,*], Mohammad Ghannadi-Maragheh[b], Akbar Anvari[c], Seyed Mahmoud Reza Aghamiri[c], Simindokht Shirvani-Arani[b], Ali Bahrami-Samani[b]

[a]*Department of Radiation Application Engineering, Shahid Beheshti University, Tehran, Iran.*
[b]*Radiopharmaceutical Research and Development Lab (RRDL), Nuclear Science and Technology Research Institute (NSTRI), AEOI, Tehran, Iran*
[c]*Department of Radiation Medicine Engineering, Shahid Beheshti University, Tehran, Iran*

Abstract

Bone is the third most common site of metastatic disease. Bone pain is the major source of morbidity associated bone metastasis. Bone-seeking radiopharmaceuticals have been applied for many years. The ability to simultaneously treat multiple sites of disease with a more probable therapeutic effect in earlier phases of metastatic disease is one of the advantages of radiopharmaceuticals. ^{175}Yb is one of the radioisotopes with suitable properties for developing various nuclear medicine agents. Some of these proper properties include 4.2 days half-life, gamma-rays emitted, radionuclidic purity. Radiopharmaceuticals capable of targeting bone tumors generally use phosphonic acid functionality as the targeting moiety. In this direction cyclic tetraphosphonate, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminomethylenephosphonate (DOTMP) has been labeled with $^{175}YbCl_3$. Production, quality control and biodistribution studies of ^{175}Yb-DOTMP were targeted in this study. ^{175}Yb chloride with mean specific activity of 31 mCi/mg was obtained by thermal neutron flux (3×10^{13} n.cm^{-2}.s^{-1}) of a natural Yb_2O_3 sample (isotopic purity of 31.8% for ^{174}Yb) in the Tehran Research Reactor (TRR). Radiolabeling was completed in one h by the addition of DOTMP at room temperature. The radiochemical purity was determined using ITLC and it was more than 98%. The results of biodistribution animal studies are excellent. It was rapidly taken up in the bone in 2 h after injection (ID/g%=3.92) and reminded after 4 d (ID/g%=3.91).

Keywords: Biodistribution; Bone Metastases; DOTMP; Radiopharmaceutical; Ytterbium-175.
Received: December 6, 2011; *Accepted:* February 19, 2012.

*Corresponding author: Laleh Safarzadeh, Department of Radiation Application Engineering, Shahid Beheshti University, Tehran, Iran, Postal code: 19839-63113.
Tel: (+98)21-22432290; Fax: (+98)21-29902546
E-mail: LaleSafarzade@gmail.com

1. Introduction

Metastatic bone disease develops as a result of many interactions between bone cells and tumor cells such as carcinomas of unknown

primary site, like lung, breast, kidney, prostate, and thyroid that often occur during the final stages of cancer and can lead to bone pain [1-3].

Methods of palliative treatment of painful bone metastases normally are nonsteroidal analgesics to opioids and chemotherapy or hormonal therapy, and radiation treatment using external-beam, sealed or unsealed sources [4]. However, many of these treatments are limited in their efficacy or duration and have significant side effects [5]. Bone-seeking radiopharmaceuticals labeled with beta emitters to relieve intense bone pain resulting from metastases have been shown to be clinically useful [6]. Substantial advantages include the ability to simultaneously treat multiple sites of disease with a more probable therapeutic effect in earlier phases of metastatic disease, the ease of administration, the repeatability, and the potential integration with other treatments [7].

Radiopharmaceuticals developed for bone pain palliation use the following radionuclides: ^{32}P, ^{89}Sr, ^{186}Re, ^{188}Re, ^{153}Sm, and ^{177}Lu [7]. The major challenge in developing effective agents for palliative treatment of bone pain arising from skeletal metastases is to ensure the delivery of adequate doses of ionizing radiation, at the site of the skeletal lesions, with minimum radiation-induced bone marrow suppression [8]. When lanthanides are administrated intravenously as salts, the main part of the dose (around 60-80%) is accumulated in the skeleton and the liver, and when lanthanides are chelated with organic ligands these molecular complexes distribute more homogeneously in the body and are essentially excreted by the kidneys in a few h [9].

^{175}Yb is one of the potential lanthanide that has suitable radionuclidic properties for developing various radiotherapy agents. ^{175}Yb decay by emission of β-particles with 470 keV maximum energy (86.5%) to stable ^{175}Lu with a convenient half-life of 4.2 days. ^{175}Yb also emits photons of 113 keV (1.9%), 282 keV (3.1%) and 396 keV (6.5%) which are appropriate for studying the biolocalization [10]. ^{175}Yb can be produced by thermal neutron bombardment of natural ytterbium target. The simplified production scheme is:

$^{174}Yb\ (n, \gamma)\ ^{175}Yb \rightarrow\ ^{175}Lu$ (Stable) σ= 69 barn

Reactions leading to the formation of radionuclidic impurities upon thermal neutron bombardment of natural ytterbium target include:

$^{168}Yb\ (n, \gamma)\ ^{169}Yb \rightarrow\ ^{169}Tm$ (Stable) σ= 2300 barn

$^{176}Yb\ (n, \gamma)\ ^{177}Yb \rightarrow\ ^{177}Lu \rightarrow\ ^{177}Hf$ (Stable) σ= 2.4 barn [11]

By attention to the presence of low amounts of ^{169}Yb in natural ytterbium target (0.13%) should not cause any serious problem in the in vivo application of ^{175}Yb. On the other hand, the presence of ^{169}Yb will be useful in extended studies of the pharmacological characteristics of the ^{175}Yb labeled radiopharmaceuticals in biological systems [12]. Also, ^{177}Lu is another radionuclidic impurity and is itself a potential therapeutic radionuclide already under investigation. The radionuclidic characteristics and chemical

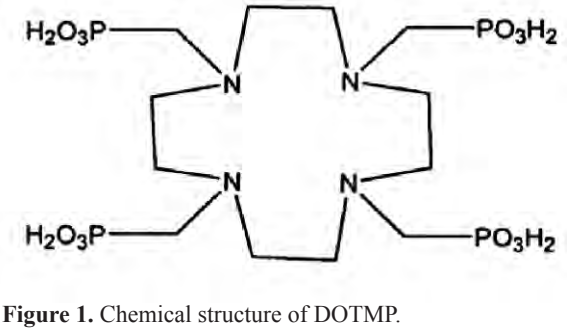

Figure 1. Chemical structure of DOTMP.

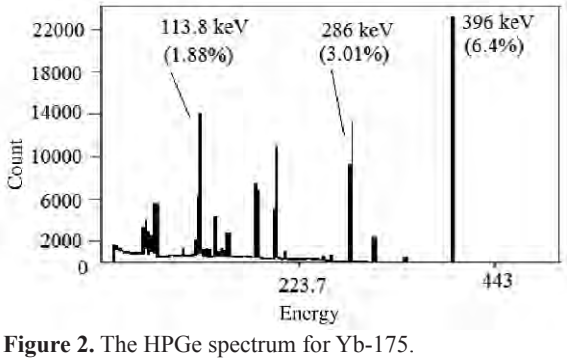

Figure 2. The HPGe spectrum for Yb-175.

properties of ^{177}Lu are very similar to ^{175}Yb. Hence, the presence of ^{177}Lu in very small quantities in the ^{175}Yb produced should not restrict the use of the latter in the in vivo therapy [13].

Multidentate aminomethylenephosphonic acids form stable complexes with different radionuclide's, and they have already proven to be very effective for palliation of bone pain [14]. The choice of using cyclic chelator is also based on the more pronounced thermodynamic stability and kinetic inertness of their lanthanide complexes when compared to that of their acyclic analog [15]. In this direction, cyclic tetraphosphonate, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminome thylene-phosphonate as DOTMP has been labeled with ^{175}YbCl3. The paper describes the successful radio labeling of this ligand with ^{175}Yb (Figure 1).

2. Materials and methods

The natural ytterbium oxide was purchased from Isotec Inc, USA and ^{175}Yb was produced in the Tehran Research Reactor (TRR). Chemical components were obtained from Sigma-Aldrich Chemical Co. U.K. All radioactivities counting related to paper chromatography were carried out using a NaI (Tl) scintillation counter on adjustment of the base line 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 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).

2.1. Production and quality control of $^{175}YbCl_3$ solution

Ytterbium-175 was produced by neutron irradiation of 1 mg of natural Yb_2O_3 at the neutron flux of 3×10^{13} n/cm^2/s. Irradiation was carried out for 7 d. The irradiated target was dissolved in 0.1 M HCl and the resultant solution was evaporated until shrivels and was reconstituted in double distilled water. The radionuclidic purity of the solution was checked using high purity germanium (HPGe) spectroscopy for the detection of various interfering gamma emitting radionuclides. The radiochemical purity of the $^{175}YbCl_3$ was checked using one solvent system of ITLC (NH_4OH: MeOH: H_2O (1:10:20)).

2.2. Labeling of DOTMP with $^{175}YbCl_3$

DOTMP solution was prepared by dissolving 10 mg of ligand in 1 ml of $NaHCO_3$ (0.5 M) at the pH 9. Then 0.3 ml of this

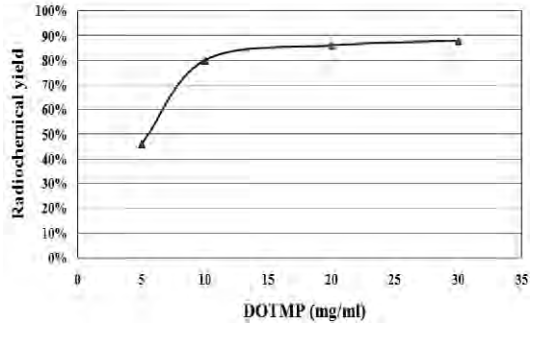

Figure 3. Radiochemical yield (RCY) of ^{175}Yb-DOTMP in radio labeling at 25°C in 24 h.

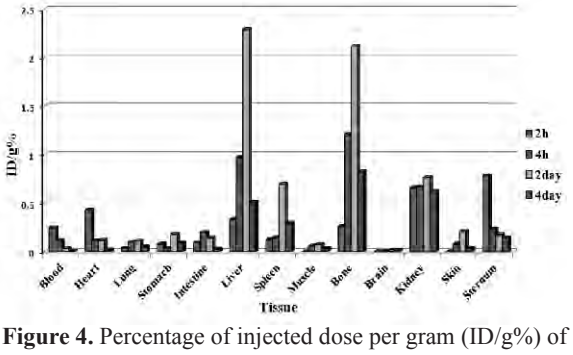

Figure 4. Percentage of injected dose per gram (ID/g%) of $^{175}YbCl_3$ in wild-type rat tissues at 2, 4 h and 2, 4 d post injection.

solution was added to 100 µl $^{175}YbCl_3$ (100 MBq). The reaction mixtures were remained with stirring at room temperature for one h. Sterility, apyrogenicity and toxicity were ascertained by routine methods.

2.3. Quality control techniques

2.3.1. Paper chromatography

For determination of the stability of complex (^{175}Yb-DOTMP), it was applied to Whatman no. 3 chromatography paper in NH_4OH: MeOH: H_2O (1:10:20) system.

2.3.2. In vitro stability studies

The stability of the complex stored at room temperature (22 ºC), fridge (4 ºC) and in the presence of freshly prepared human serum (at 37 ºC) was checked at different time points by paper chromatography in NH_4OH:MeOH: H_2O (1:10:20) system to determine the radiochemical purity of the radiolabeled complex.

2.3.3. Biodistribution studies

Biodistribution studies of ^{175}Yb complex were carried out in Wistar rats. Rat's weight was 170-220 g and two rats were sacrificed for each time point. The complex solutions (0.15-0.2 ml; 160-180 µCi) were injected through the tail vein of the rats. For comparison, free Yb^{3+} cation buffer solution was also administered. Briefly, 0.15-0.2 ml of final ^{175}Yb-Cl_3 solution with 160-180 µCi was injected intravenously to rats, too. The animals were sacrificed post-anesthesia at 2, 4 h and 2, 4 d post-injection. The tissues and the organs were excised and the activity associated with each organ was measured in a NaI(Tl) scintillation counter. The distributed activity in different organs was determined by calculation as the percentage of the injected activity (based on area under the curve of 396 keV peak) per gram of the organ. The institutional and international guide for the care and use of laboratory animals were followed.

3. Results and discussion

3.1. Production of 175Yb

Around 1.3-1.5 GBq/g (35-40 Ci/g) of ^{175}Yb activity was obtained after 7 days irradiation at a flux of 3×10^{13} n/cm^2/s using natural Yb_2O_3 target. Other result of this irradiation is also ^{169}Yb and ^{177}Lu as radionuclidic impurities. The gamma-ray spectrum of irradiated target after chemical processing is shown in Figure 2. The observed gamma-photo peaks correspond to the gamma-photo peaks of ^{175}Yb (113, 144, 286 and 396 keV), ^{169}Yb (63, 110, 130, 177, 198, 261 and 307 KeV) and ^{177}Lu (208 and 250 keV). By analyzing the gamma-ray spectra, the radionuclidic purity of ^{175}Yb was found to be 96.2% with the presence of 2.1% ^{169}Yb

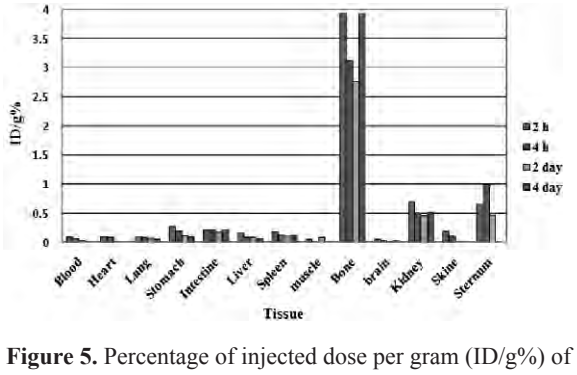

Figure 5. Percentage of injected dose per gram (ID/g%) of ^{175}Yb-DOTMP in wild-type rat tissues at 2, 4 h and 2, 4 d post injection.

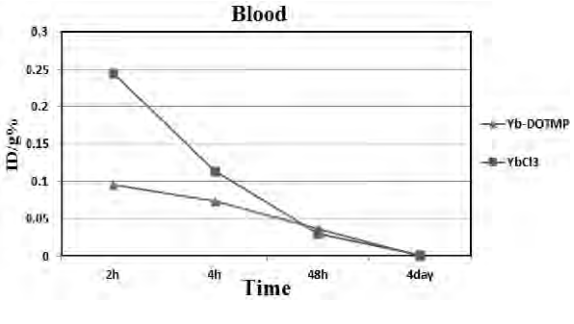

Figure 6. Comparative blood activity for ^{175}Yb-DOTMP and $^{175}YbCl_3$ in wild-type rats.

and 1.7% ^{177}Lu as radionuclidic impurities.

3.2. Labeling optimization studies

To obtain the highest labeling yield, quantitative studies were performed. In this study, different amounts of the ligand for a specific amount of radioactivity (2.8 mCi of $^{175}YbCl_3$ for instance) was used in a suitable temperature (25 °C). The labeling yield of 45-88% was obtained at room temperature using different amounts of the ligand within 24 h (Figure 3).

3.3. Stability of ^{175}Yb-EDTMP in final formulation

By attention to optimized reaction conditions the stability of the ^{175}Yb-DOTMP complex was studied and was observed that the complex has excellent stability when stored at room temperature. The complex remained stable to the extent of 88% up for 96 h, whereas stability of this compound was shown 90% for 72 h in refrigerator. The free ytterbium cation in $^{175}Yb^{3+}$ form remains at the origin (Rf= 0.0) and the ^{175}Yb-DOTMP complex migrates to higher Rf (Rf>0.88).

3.4. Biodistribution of ^{175}Yb cation and ^{175}Yb-DOTMP in rats

For substantiating the significant accumulation of ^{175}Yb-DOTMP in bone, it is necessary to perform a comparison between ^{175}Yb-DOTMP and free ytterbium cation biodistribution data. Thus the biodistribution of the cation was checked in various vital organs after injection of 6-7 MBq of the $^{175}YbCl_3$ pre-formulated by the normal saline (pH= 8) to each rat.

On the other hand, a volume (0.2 ml) of ^{175}Yb-DOTMP solution with 0.16-0.18 mCi activity and pH=8-9 was injected intravenously to each rat through tail vein. The animals were sacrificed at the exact time intervals (2, 4, 48 h and 4 d), and specific activity of different organs was calculated as percentage of injected dose per gram using NaI(Tl) detector (Figures 4 and 5).

The Liver uptake of the free ^{175}Yb cation is relatively high. About 2.3% of the activity accumulates in the liver after 2 days. Beside the kidney was one of the major accumulation sites of the radiolabeled DOTMP. Therefore, it can be concluded that free ^{175}Yb is extracted from the liver due to free cation release through the biliary tract, while in case of ^{175}Yb-DOTMP the uptake reaches its maximum at 2 h followed by excretion. Lung, muscle and also skin do not demonstrate significant uptake which is in accordance with other cations accumulation.

For demonstration of ligand effect in organs uptake can be compared of ^{175}Yb-DOTMP and $^{175}YbCl_3$ behavior in wild-type rat tissues. By attention to Figure 6 it can be realized for ^{175}Yb-DOTMP the blood content is low at all time intervals and this shows the

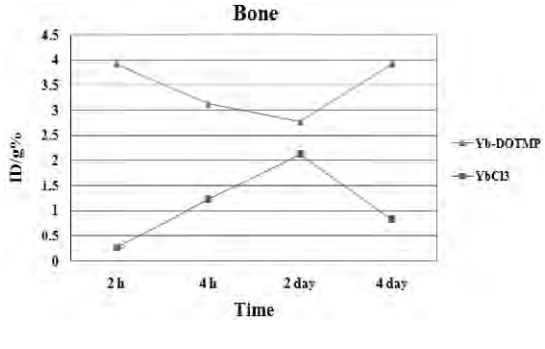

Figure 7. Comparative bone activity for ^{175}Yb-DOTMP and $^{175}YbCl_3$ in wild-type rats.

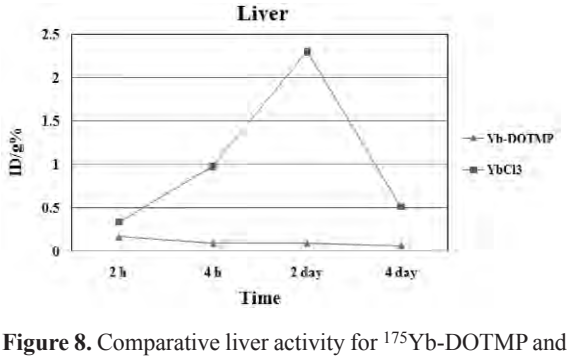

Figure 8. Comparative liver activity for ^{175}Yb-DOTMP and $^{175}YbCl_3$ in wild-type rats.

rapid removal of activity in the circulation. Toward $^{175}YbCl_3$ the activity in blood is in the highest value at first two hours and with different mechanism was washed out from the circulation after 4 days.

A 2.12% bone uptake is observed for the cation in 2 days after injection and then decreased (Figure 7). On the other hand, ^{175}Yb-EDTMP complex was rapidly taken up in the bone in 2 h after injection (ID/g%= 3.92) and remained almost constant after 4 days (ID/g%=3.91)

^{175}Yb cation is accumulated in the liver in the 2 days post injection, and it can be assumed that later the activity is excreted from liver. But liver uptake for ^{175}Yb-DOTMP is negligible (Figure 8).

4. Conclusion

It was observed from the animal tests and quality control data of ^{175}Yb-DOTMP that is shows good features to be used as bone pain palliation again. Quality control and animal tests data of ^{175}Yb-DOTMP show good features to be used as bone pain palliation agent.^{175}Yb-DOTMP complex was prepared and was carried out quality control using optimized condition. For ^{175}Yb-DOTMP, radiochemical purity was higher than 98%, also radionuclidic purity was acceptable. The labeling and quality control took one hour and radiolabeled complex was stable in human serum for least 2 days. The biodistribution data on normal rats showed at least 4% uptake of ^{175}Yb-DOTMP is in gram of the bone tissues. The produced ^{175}Yb-DOTMP properties such as relatively long half-life, appropriate beta and gamma energy, low cost and easy production suggest good potential for efficient use of this radiopharmaceutical for bone pain palliation of skeletal metastases.

Acknowledgment

The authors would like to thank Dr. Majid Shahriari for his guidance and supervision when he was with us. God bless his soul.

References

[1] Coleman R. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev* 2001; 27: 165-76.

[2] Clines G, Guise T. Mechanisms and treatment for bone metastases. *Clin Adv Hematol Oncol* 2004; 2: 295-302

[3] Casciato DA, Territo MC. Manual of clinical oncology, Lippincott Williams & Wilkins; London;2008:151-3.

[4] Hillegonds DJ, Franklin S, Shelton DK, Vijayakumar S, Vijayakumar V. The management of painful bone metastases with an emphasis on radionuclide therapy. J *Natl Med Assoc* 2007; 99: 785-94

[5] Serafini AN. Therapy of metastatic bone pain. *J Nucl Med* 2001;42:895-906.

[6] Bodei L, Lam M, Chiesa C, Flux G, Brans B, Chiti A, Giammarile F. EANM procedure guideline for treatment of refractory metastatic bone pain. Eur *J Nucl Med* Mol Imag 2008; 35: 1934-40.

[7] Pandit-Taskar N, Batraki M, Divgi CR. Radiopharmaceutical therapy for palliation of bone pain from osseous metastases. *J Nucl Med* 2004; 45:1358-65.

[8] Chakravarty R, Das T, Venkatesh M, Dash A. An electro-amalgamation approach to produce ^{175}Yb suitable for radiopharmaceutical applications. Radiochim Acta 2012;100:4:255-61.

[9] Taylor D, Leggett R. A generic biokinetic model for predicting the behaviour of the lanthanide elements in the human body. Rad Protec Dosimet 2003; 105: 193-8

[10] Firestone RB, Chu S, Baglin CM. of the Table of Isotopes: 1998 Update. In: editor.^editors.: 1997. p.

[11] Chakraborty S, Unni P, Venkatesh M, Pillai M. Feasibility study for production of 175Yb: a promising therapeutic radionuclide. Appl Rad Isotopes 2002; 57: 295-301.

[12] Mathew B, Chakraborty S, Das T, Sarma H, Banerjee S, Samuel G, Venkatesh M, Pillai M. 175Yb labeled polyaminophosphonates as potential agents for bone pain palliation. Appl Rad Isotopes 2004; 60: 635-42.

[13] Das T, Chakraborty S, Unni P, Banerjee S, Samuel G, Sarma H, Venkatesh M, Pillai M. 177Lu-labeled cyclic polyaminophosphonates as potential agents for bone pain palliation. Appl Rad Isotopes 2002; 57: 177-84.

[14] Volkert WA, Hoffman TJ. Therapeutic radiophar-

maceuticals. Chem Rev 1999; 99: 2269-92.
[15] Caravan P, Ellison JJ, McMurry TJ, Lauffer RB. Gadolinium (III) chelates as MRI contrast agents: structure, dynamics, and applications. Chem Rev 1999; 99: 2293-352.