Original Article

Production of Holmium-166 DOTMP: A promising agent for bone marrow ablation in hematologic malignancies

Reza Bagheri[1,2], Amir Reza Jalilian[1], Ali Bahrami-Samani[1], Mohammad Mazidi[1], Mohammad Ghannadi-Maragheh[1]

[1]Radiopharmaceutical Research and Development Laboratory, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Tehran, Iran.
[2]Faculty of Nuclear Engineering and Physics, Amirkabir University of Technology, Tehran-Iran.

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ABSTRACT

Intoduction: Therapeutic radiopharmaceuticals are radiolabeled molecules to deliver sufficient doses of ionizing radiation to specific disease sites such as bone metastases, brain and liver tumors and bone marrows malignancies including multiple myeloma. Among some therapeutic radiopharmaceuticals, ^{166}Ho-1,4,7,10 – tetraazacyclo dodecane–1,4,7,10 tetraethylene phosphonic acid (^{166}Ho-DOTMP) is used for delivering high doses to bone marrow. In this research production, quality control, pharmacokinetics and biodistribution studies of ^{166}Ho-DOTMP with respect to its radiochemical and in vivo biological characteristics have been presented.
Methods: Holmium-166 was produced by irradiation of holmium oxide (Ho2O3, purity > 99.8%) at a thermal neutron flux. ^{166}Ho-DOTMP complex was obtained in very high yields (radiochemical purity > 99%) under the reaction conditions employed. Radiochemical purity and the stability of the ^{166}Ho-DOTMP complex in human serum were assayed. Wild type rats were used for biodistribution and imaging studies of this agent.
Results: ^{166}Ho produced by irradiation of holmium-165 oxide demonstrated high radionuclide purity. ^{166}Ho-DOTMP was obtained in very high yield (radiochemical purity > 99%) and the complex exhibited excellent in vitro stability at pH~7 when stored at room temperature and human serum. Biodistribution studies in rats showed favorable selective skeletal uptake with rapid clearance from blood along with insignificant accumulation of activity in other non-target organs. The scintigraphic image recorded in rat at 3 h after the injection of the ^{166}Ho-DOTMP radiopharmaceutical revealed that ^{166}Ho-DOTMP rapidly accumulated in skeleton especially in the thigh bones.
Conclusion: Biodistribution, stability, imaging and pharmacokinetics studies of ^{166}Ho-DOTMP radiopharmaceutical in this research showed favorable features such as; rapid and selective skeletal uptake, fast clearance from blood and almost no uptake in any other major organs. Our research demonstrated that ^{166}Ho-DOTMP has promising features suggesting good potential for efficient use of this radiopharmaceutical for bone marrow ablation in different hematologic malignancies including multiple myeloma.

Keywords: ^{166}Ho-DOTMP, Biodistribution, radiopharmaceutical, pharmacokinetics.

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Corresponding author: Dr Amir Reza Jalilian, Radiopharmaceutical Research & Development Laboratory, Nuclear Science and Technology Research Institute, AEOI, Tehran, Iran
E-mail: ajalilian@nrcam.org

INTRODUCTION

Multiple myeloma and other hematological malignancies have been treated by myeloablative radiotherapy/chemotherapy and subsequent stem cell transplantation. Currently, there is interest in the selective delivery of radiation to tumor cells and bone marrow to increase efficacy and reduce morbidity. ^{166}Ho- 1, 4,7,10 – tetraazacyclo dodecane – 1, 4,7,10 tetra ethylene phosphonic acid (^{166}Ho-DOTMP) (Figure 1) and ^{153}Sm-ethylenediaminetetramethylene phosphonate (^{153}Sm-EDTMP) have been proposed as bone-seeking radiopharmaceuticals to deliver an ablative radiation dose to marrow while minimizing non hematological toxicity (1-3).

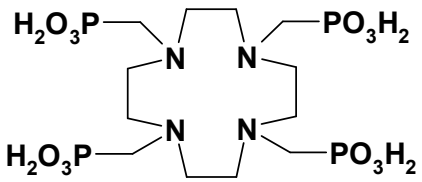

Fig 1. Chemical formula for DOTMP

^{166}Ho-radiopharmaceuticals have been also developed and used in the therapy of various diseases and malignancies. Holmium-166 microspheres are widely used for the treatment of liver malignancies, gliomas and prostate cancer (4).

^{166}Ho-ethylenediaminetetramethylene phosphonic acid (EDTMP) is used to treat pain when cancer has spread to the bone. It homes on areas where cancer has invaded the bone and emits beta particles which kill the nearby cancer cells (5, 6). This radionuclide has also been extensively used in radiation synovectomy in form of ^{166}Ho-macroaggregates to destroy the inflamed synovium (7).

Holmium-166 has relatively high beta energy ($E_{\beta,max}$= 1.84 MeV, $E_{\beta,ave}$= 0.67 MeV), long penetration range in tissue (this is why holmium-166 is used for larger tumors), gamma ray (~ 81 keV, 6.7%) for scintigraphic imaging studies and approximately short half-life ($T_{1/2}$= 1.1 d) for delivering high doses in short period of time (8).

In this research we describe the preparation and quality control of ^{166}Ho-DOTMP. Biodistribution and pharmacokinetic studies of the complex is investigated vital rat imaging as well as organs distribution in rat.

METHODS

Holmium oxide (purity > 99.8 %) as the target at the Tehran Research Reactor (TRR) using ^{165}Ho (n,γ) ^{166}Ho nuclear reaction with purity of >99.99% was obtained from ISOTEC Inc. DOTMP and other chemicals were purchased from Fluka Co. Switzerland. Whatman No. 1 paper was used for paper chromatography. The counting of organs and chromatography papers as well as the determination of radionuclide purity of ^{166}Ho was carried out by high resolution gamma-ray spectrometry using an HPGe detector on the base of 80.75 keV peak and beta-particle spectrometry using the Wallac 1220 Quantulus liquid scintillation spectrometer. Scintigraphic images were obtained using a dual-head SPECT system.

Production and quality control techniques of $^{166}HoCl_3$

About 80 mCi of ^{166}Ho activity was obtained at 11 h post bombardment for 1mg of natural holmium-165 oxide for 36 hour at a thermal neutron flux of 3.5×10^{13} n.cm^{-2}.s^{-1} corresponding to a specific activity of 80 mCi/mg. The irradiated target was dissolved in 1 ml of 0.1 N HCl to prepare $^{166}HoCl_3$. The radionuclide purity of the solution was checked by beta spectroscopy as well as HPGe spectroscopy. The radiochemical

purity of the $^{166}HoCl_3$ was checked using two solvent systems for ITLC, A: 10 mM DTPA solution (pH~ 4) and B: 10% ammonium acetate: methanol (1:1).

Radiolabeling and molar ratio studies of DOTMP with $^{166}HoCl_3$

DOTMP was dissolved in 2 N NaOH solution and diluted to the appropriate volume with ultra pure water to produce a solution of 50 mg/ml DOTMP solution that pH was adjusted to 7.5-8. To the 0.75 ml of resulting solution, containing the 37.5 mg (72.3 µM) of DOTMP, 0.25 ml of $^{166}HoCl_3$ solution (700-750 MBq of ^{166}Ho activity) containing 0.25 mg (1.515 µM) of Ho was added following the addition of 0.5 ml phosphate buffer (pH=8). The pH of the final solution was adjusted to 7-8. The complex solution was incubated at room temperature for 1 h. The radiolabeling yield and radiochemical purity were determined by paper chromatography using ammonia: methanol: water (1:10:20) as the eluting solvent and both the Whatman No. 1 Paper and SG sheet as the stationary phase. For optimization of the labeling yield experiments were carried out to determine the complexation yields of ^{166}Ho-DOTMP at different [ligand]:[metal] ratios ranging between 1:1 to 1:50 by varying the ligand amount while keeping the amount of Ho fixed at 0.25 mg.

In vitro stability studies

The In-vitro stability of the ^{166}Ho-DOTMP was studied by storing the complex at room temperature at pH~7.5 for a period of 72 h after preparation. The radiochemical purity of the complex was assessed at regular time intervals by paper chromatography using system mentioned above. The stability of the ^{166}Ho-DOTMP complex in human serum was also assayed. 50 µl of the complex was added to 0.5 ml of human serum and the mixture was incubated at 37 °C. The radiochemical purity was determined by employing paper chromatography. The stability of the ^{166}Ho-DOTMP complex was also studied in 0.1 M HCl medium at room temperature because H^+ strongly competes with free holmium cation in $^{166}Ho^{3+}$ form for the DOTMP ligand.

Biodistribution studies of ^{166}Ho-DOTMP in rats

After the preparation of ^{166}Ho-DOTMP, final complex solution was passed through a 0.22 µm membrane filter and diluted. Approximately 150 µl complex solution (pH~7.5) containing 150 ± 5 µCi of radioactivity was injected through the tail vein and the animals were sacrificed at the end of 2, 4, 24, 48, 66 h post-injection. For better comparison of the ^{166}Ho-DOTMP and $^{166}HoCl_3$ species behavior and pharmacokinetic studies of this radiopharmaceutical, the biodistribution pattern of $^{166}Ho^{3+}$ as well was determined in wild type rats by injection of 150 µl (150 ± 5 µCi of ^{166}Ho) $^{166}HoCl_3$ solution through tail vein. The tissues and the organs were excised and the associated specific activity was measured by gamma spectrometer with an HPGe detector.

Imaging studies in rats

For scintigraphic imaging studies 150 µl (150 ± 5 µCi) of the ^{166}Ho-DOTMP solution was injected through the tail veins of the rats followed by propofol- xylazine mixture injection for anaesthetization. The images were taken at 3 h post-injection using dual-head SPECT system. The SPECT system was previously calibrated for 80.75 keV peak by the ^{133}Ba (15% energy window). The rat-to-septa distance was 12 cm. The useful field of view (UFOV) was 540 mm × 400 mm. The spatial resolution was 10 mm FWHM at the CFOV. All the images were recorded by acquiring 500 k counts using 256 × 256 matrix sizes.

RESULTS

Production and quality control techniques of $^{166}HoCl_3$

^{166}Ho was produced by irradiation of 1mg of $^{165}Ho_2O_3$ (purity > 99.8%) with a specific activity of 80-85 mCi/mg. Radionuclide purity of ^{166}Ho was checked by two spectrometry system, A: The gamma-ray spectrum of the irradiated material showed the characteristic gamma rays of ^{166}Ho: 80.68 keV and 1379.94 keV which are identical to published nuclear data (Figure 2). B: Beta spectroscopy depicted a continual spectrum with no unexpected isotopic impurity (Figure 3).

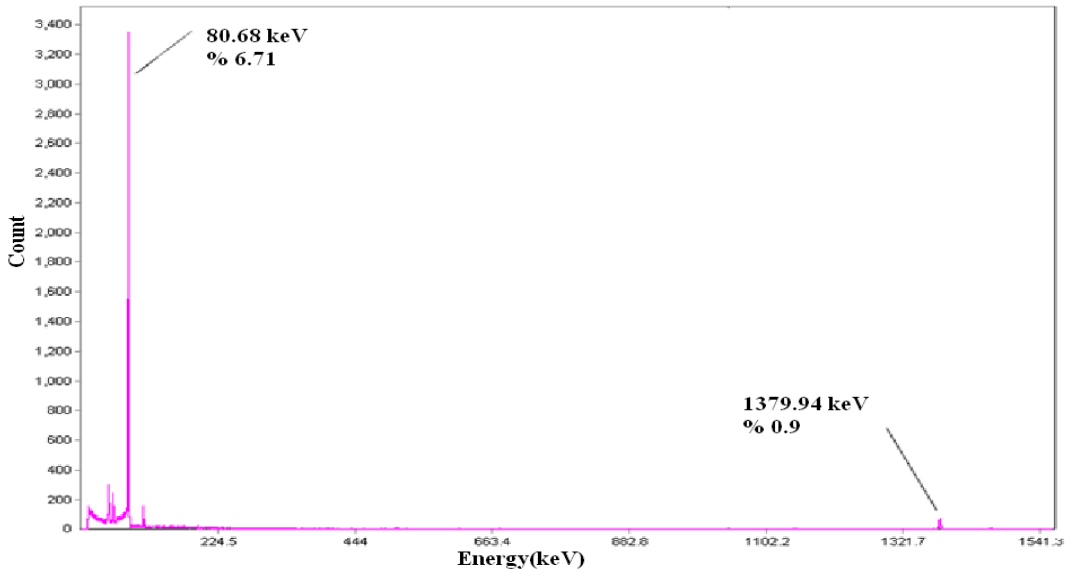

Fig 2. Gamma spectrum for $^{166}HoCl_3$ solution.

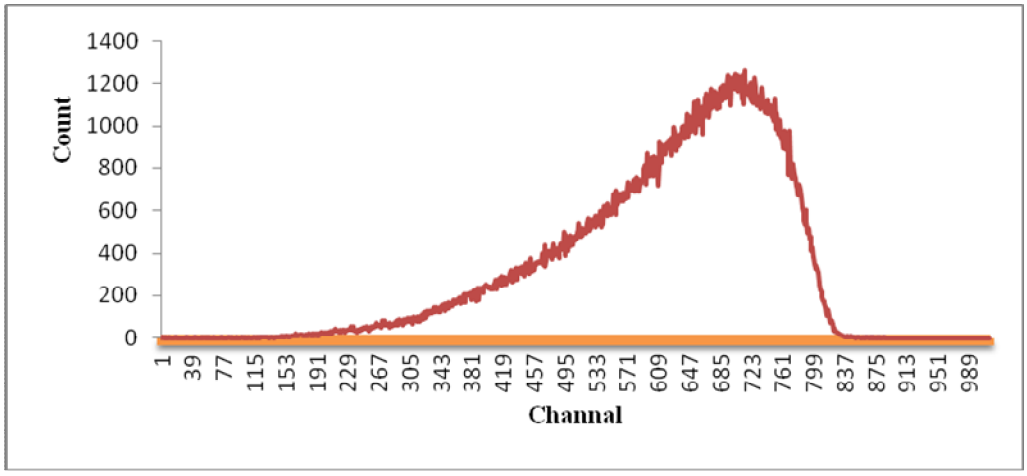

Fig 3. Beta spectrum for $^{166}HoCl_3$ solution prepared by neutron irradiation of ^{165}Ho sample using a liquid scintillation counter.

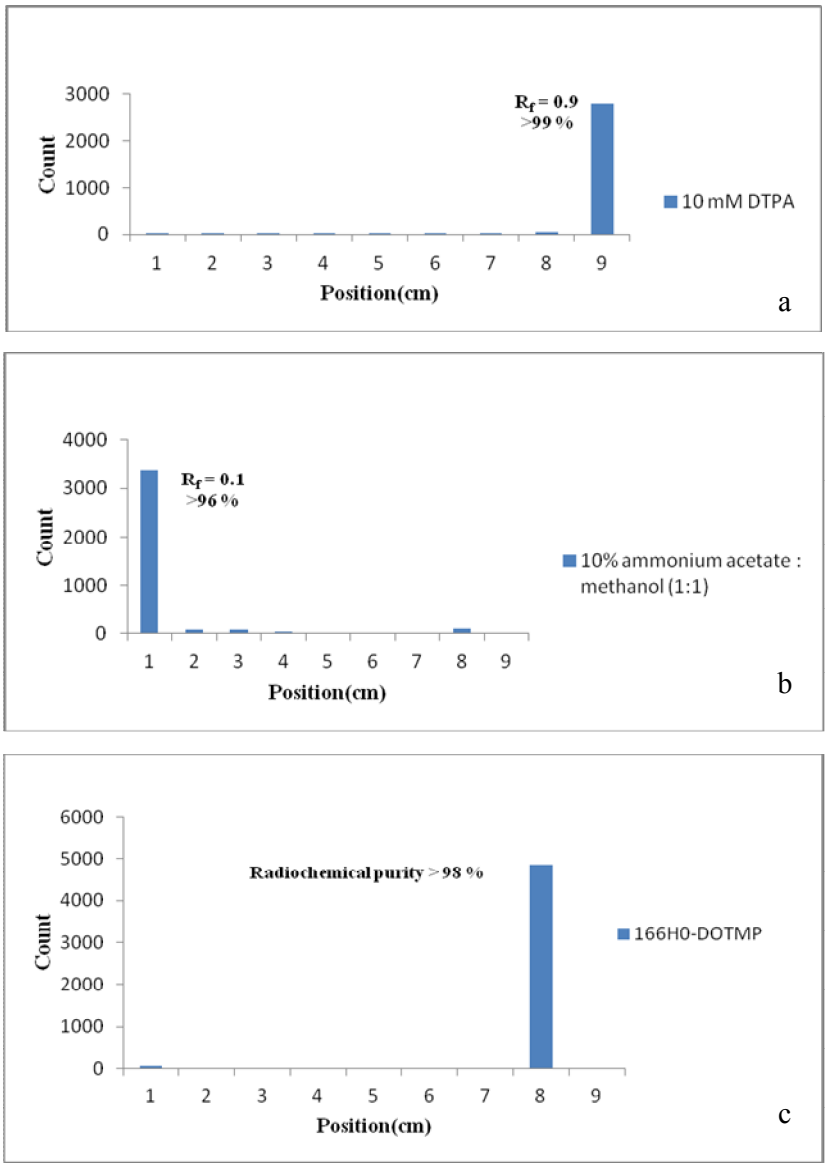

Fig 4. ITLC chromatograms of $^{166}HoCl_3$ solution in; 10 mM DTPA solution (pH~ 4) (a), 10% ammonium acetate:methanol (1:1) (b); ^{166}Ho-DOTMP complex radiochromatogram eluted with ammonia:methanol:water (1:10:20) (c) on Whatman No. 1 Paper.

Radiochemical purity of the $^{166}HoCl_3$ solution were checked by two solvent systems for ITLC, A: 10 mM DTPA solution (pH~ 4) as mobile phase on Whatman No. 1 paper, the free holmium cation in $^{166}Ho^{3+}$ form, was chelated with the polydentate compound leading to the migration of the cation in ^{166}Ho-DTPA form to higher R_f (R_f = 0.9), any other ionic species (such as $^{166}HoCl_4^-$, etc.) would lead to the observation of new radiopeaks, especially at the origin (R_f = 0.1).

B: 10% ammonium acetate: methanol (1:1) was used as another solvent system on the Whatman No. 1 paper, $^{166}Ho^{3+}$ remains at the origin using this system while other ionic species would migrate to higher R_fs (Figure 4).

Radiochemical purity and molar ratio studies of DOTMP with $^{166}HoCl_3$

^{166}Ho-DOTMP was obtained in very high yield (radiochemical purity > 99%). Table 1 shows the complexation yield of ^{166}Ho-DOTMP obtained at different [ligand]:[metal] ratio. It was observed that the complex was obtained in very high yield (> 98%) when the [ligand]: [metal] ratio was 30:1 or higher. It was observed that at higher molar ratios the liver uptake increases, therefore the molar ratio of 40:1 was chosen for in-vivo studies.

Table 1. Complexation yield of ^{166}Ho-DOTMP at different [ligand]:[metal] ratios.

Molar ratio [Ligand]:[metal]	Complexation yield (%)
50:1	99.11
40:1	98.96
30:1	98.23
10:1	94.29
5:1	91.94
2:1	91.84
1:1	82.63

In paper chromatography using ammonia : methanol : water (1:10:20) as the eluting solvent and both the Whatman No. 1 Paper and Silica-Gel sheet (1.5 cm ×10 cm) as the stationary phase, ^{166}Ho-DOTMP complex moved toward the solvent front (R_f = 0.8) while uncomplexed $^{166}HoCl_3$ remained at the point of spotting (R_f = 0.1) under identical conditions.

Stability studies

^{166}Ho-DOTMP complex exhibited excellent in-vitro and in-vivo stability at pH ~ 7 when stored at room temperature and in human serum at 37 °C. The radiochemical purity of both conditions was found to be retained to extend of > 97% after 72 h post-preparation. When the complex was studied in 0.1 M HCl medium (pH~ 2) at room temperature, it was found that ^{166}Ho-DOTMP complex undergo significant decomposition to the extent of < 27% within 48 h. (Figure 5).

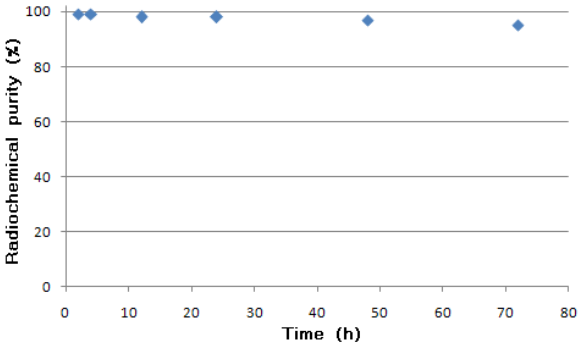

Fig 5. Stability of the complex in final solution up to 72 h (pH. 5.5-7).

Biodistribution studies of ^{166}Ho-DOTMP in wild type rats

The animals were sacrificed by CO_2 asphyxiation at selected times after injection. The uptake of ^{166}Ho-DOTMP complex in different organs or tissues of wild type rats was calculated as percentage of the injected dose per gram organs or tissues of rats (ID/g %). The results of the biodistribution studies revealed significant bone uptake (target tissue) within 2 h post-injection. Almost all the activity from blood was cleared within 4 h post-injection, only barring small uptake in liver and kidney was observed which are the important target tissues for the free holmium cation in $^{166}Ho^{3+}$ form (Figure 6).

For $^{166}HoCl_3$ solution, the radioactivity (the free holmium cation in $^{166}Ho^{3+}$ form) was mainly located in the liver, kidney, and bone (Figure 7). Both compounds are washed out from the circulation after 48 h, although the blood wash-out mechanisms are different.

Figure 8 demonstrates the bone uptake from 2 to 48 h. ^{166}Ho-DOTMP is rapidly taken up in bones in 2 h after administration and reached almost % 3.5 up to 48 h. however, $^{166}Ho^{3+}$ cation uptake slowly increased up to 4 h then decreased to % 0.75.

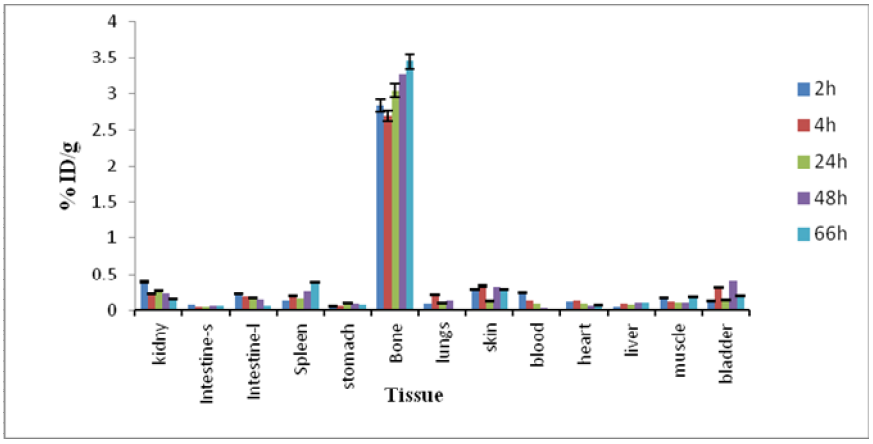

Fig 6. Percentage of injected dose per gram (%ID/g) of ^{166}Ho-DOTMP in wild type rat tissues at 2, 4, 24, 48 and 66 h post-injection.

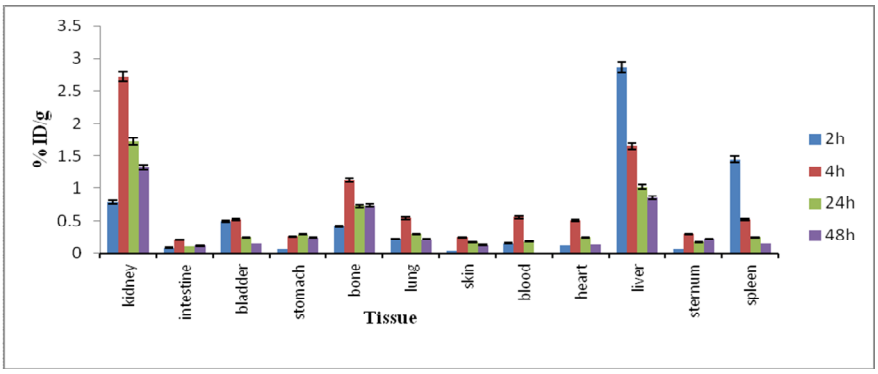

Fig 7. Percentage of injected dose per gram (%ID/g) of $^{166}HoCl_3$ in rat tissues at 2, 4, 24 and 48 h post injection.

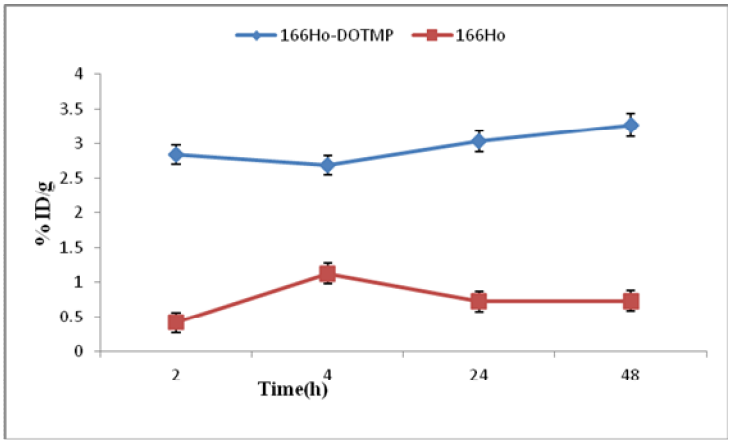

Fig 8. Comparative bone activity for ^{166}Ho-DOTMP and $^{166}HoCl_3$ in wild type rats.

^{166}Ho-DOTMP is rapidly taken up in bones and retains almost constant up to 48 h. Instead, as a water soluble cation most of free $^{166}Ho^{3+}$ activity is washed out through kidney in 48h.

^{166}Ho-DOTMP has almost no liver accumulation, which is a major advantage as a therapeutic radiopharmaceutical due to the possibility of increasing the maximum administered dose compared to other bone seeking therapeutic radiopharmaceuticals such as ^{177}Lu-EDTMP and ^{153}Sm-EDTMP. Since $^{166}Ho^{3+}$ cation, being transferred by serum metalloproteins, accumulates in liver and is excreted through hepatobilliary excretion route, leading to the reduction in liver accumulation.

A major difference in spleen uptake similar to liver uptake is observed for the two species in the early hours after administration. ^{166}Ho-DOTMP almost is not accumulated in spleen more than % 0.25 up to 48 h after injection while $^{166}Ho^{3+}$ cation accumulated in spleen almost % 1.5 in the early hours then decreased to % 0.14 after 48h.

Imaging studies in rats

The scintigraphic image recorded in rat at 3 h after the injection of the ^{166}Ho-DOTMP radiopharmaceutical revealed that ^{166}Ho-DOTMP rapidly accumulated in skeleton especially in the thigh bones. The residual activity underwent rapid renal excretion from the circulation in first few hours through kidneys and bladder initially which was found to diminish gradually with the progress of time (Figure 9).

DISCUSSION

^{166}Ho-DOTMP radiopharmaceutical was readily obtained in high [ligand]:[metal] ratio (30:1) (Table 1).This agent was prepared with high complexation yield (radiochemical purity > 98 %) when a [ligand]:[metal] ratio was higher than 30:1, this is an important feature of the DOTMP, since it indicates superior complexing ability of this ligand. ^{166}Ho-DOTMP radiopharmaceutical demonstrated superior in-vitro stability at room temperature as it retained > 97 % radiochemical purity even after 72 h of preparation when the [ligand]:[metal] ratio was 40:1.

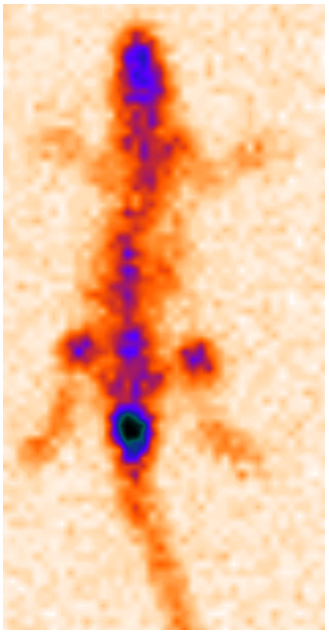

Fig 9. SPECT image of ^{166}Ho-DOTMP 3 h post-injection in wild type rat.

This has been already shown for most of the other bone pain palliation agents such as ^{153}Sm-EDTMP, in that case the ratio is almost in the range of 1:50 which retains almost the same toxicity to the both complexes (9), while in case of Ho-166 EDTMP the ratio of ligand to the activity was 1:15 (6), which is a good ratio for a bone pain palliative agent, however ^{166}Ho-DOTMP is mostly used for bone marrow ablation due to the rapid dose rate and higher injected dose.

Biodistribution studies in rats demonstrated favorable features such as; significant skeletal accumulation and rapid blood clearance. Scintigraphic image of rat

recorded after the administration of the ^{166}Ho-DOTMP showed that the radiopharmaceutical majorly trapped in bones while it washed out from circulation in first few hours through kidneys.

Interestingly the bone uptake for ^{166}Ho-DOTMP and ^{153}Sm-EDTMP is almost the same after 48 h (6), while in case of ^{166}Ho-EDTMP it is lower (2.5%). Compared to other bone agents, the present ligand, ^{166}Ho-DOTMP, is capable of imposing rather high radiation dose to the bone marrow as already shown in the literature (2, 3).

^{166}Ho-DOTMP is a promising agent for bone marrow ablation in hematologic malignancies including multiple myeloma owning to its suitable nuclear decay characteristics and easy and practical stages of production and radiolabeling of this radiopharmaceutical.

CONCLUSION

Biodistribution, stability, imaging and pharmacokinetics studies of ^{166}Ho-DOTMP radiopharmaceutical in this research showed favorable features such as; rapid and selective skeletal uptake, fast clearance from blood and almost no uptake in any other major organs. Our research demonstrated that ^{166}Ho-DOTMP has promising features suggesting good potential for efficient use of this radiopharmaceutical for bone marrow ablation in hematologic malignancies such as multiple myeloma. The radiopharmaceutical has already demonstrated successful therapeutic applications in the world. In this study, the production for the first time in the country has been reported. Clinical trial studies are under consideration by selected clinical centers.

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