Development of $^{153}Sm/^{177}Lu$-EDTMP as a possible therapeutic complex

Original Article

Hassan Ranjbar[1], Ali Bahrami-Samani[1], Davood Beiki[2], Mohammad Ghannadi-Maragheh[1]

[1] Radiopharmaceutical Research and Development Lab (RRDL), Nuclear Science and Technology Research Institute (NSTRI), P.O. Box 14395-836, Tehran, Iran
[2] Research Center for Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Introduction: Targeted radionuclide therapy (TRT) has been demonstrated to be an effective therapeutic tool in patients with disseminated bone metastasis. TRT is generally performed with a single radionuclide. In this study we investigated the feasibility of combined TRT with a high-energy beta emitter (^{153}Sm) and a low energy beta emitter (^{177}Lu) in wistar rats.

Methods: The cocktail complex of $^{153}Sm/^{177}Lu$-EDTMP was prepared. To determine the effect of metal-to-ligand (Me:EDTMP) molar ratio on labeling yield, several complex were analyzed after changing Me:EDTMP molar ratio from 1:1 to 1:50. $^{153}Sm/^{177}Lu$-EDTMP was administered intravenously through the tail vein of wistar rats. Biodistribution data were collected at 2 hours to 7 day post injection and scintigraphic images were taken at 24 hours and 1, 2 week after administration of radiopharmaceutical.

Results: The results revealed high skeletal uptake (3.5% and 3.4% ID/g at 24 hours post injection for ^{153}Sm and ^{177}Lu, respectively) with rapid blood clearance and minimal uptake in any of the major organs. Scintigraphic images verified high skeletal uptake.

Conclusion: Our results indicate that the combination of ^{153}Sm and ^{177}Lu is feasible and safe. This study suggests that the combination of different radionuclides with different radiation energies and half-life, such as ^{153}Sm and ^{177}Lu, could be advantageous in patients with tumoral lesions of different sizes.

Key words: $^{153}Sm/^{177}Lu$-EDTMP; Radiopharmaceutical cocktail; Biodistribution; Radiolabeling; Bone pain palliation

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Corresponding author: Dr. Hasan Ranjbar, Radiopharmaceutical Research and Development Lab (RRDL), Nuclear Science and Technology Research Institute (NSTRI), P.O. Box 14395-836, Tehran, Iran. E-mail: hranjbar@aeoi.org.ir

INTRODUCTION

Metastasis is largely implicated in cancer aggressiveness and is a very common and often painful experienced by many cancer patients. It is responsible for more than 90% of fatality as documented in patients with solid tumors [1, 2]. Metastasis is a complex event leading to the formation of new tumoral sites arising from a primary tumor [3, 4]. In advanced stages, these are frequently associated with adverse clinical effects including pain, fractures, and hypercalcemia causing significant morbidity affecting functional status and quality of life [5].

Radiopharmaceuticals have a vital role in the treatment of patients with multiple metastatic lesions. Bone-seeking radiopharmaceuticals play an important role in reduction of pain from bone metastases [6].

Bone-targeted radionuclide therapy (BTRT) with agents such as Strontium-89 (^{89}Sr), or radiolabelled bisphosphonates with Samarium-153 (^{153}Sm), Rhenium-186 (^{186}Re) and Rhenium-188 (^{188}Re) may be effective in bone metastatic disease, predominantly in prostate and breast cancer patients [7-14]. These kinds of therapy do not have major limitations of other therapies, such as chemotherapy and external beam radiotherapy instead demonstrate many advantages including the ability to treat multiple sites of tumoral involvement simultaneously and lack of significant conflict with other treatments [15].

EDTMP or ethylene diamine tetra methylene phosphonic acid is nitrogenous, polyphosphonic acid chelator that complexes with various radiometals, particularly lanthanides (Figure 1). all the complexes have excellent pharmacological characteristics including rapid blood clearance and high bone affinity [16, 17].

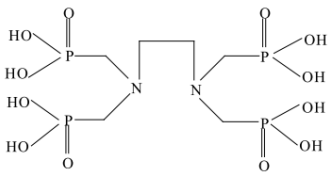

Fig 1. Structure of EDTMP

The current practice in treatment of bone metastasis utilizes a single radioisotope such as ^{153}Sm or ^{177}Lu [18-21]. ^{177}Lu emits beta particles with a low maximum energy ($E_{\beta,max}$ = 0.497 MeV) and short maximum particle range in tissues that allows concentration of most of its dose in small metastases, whereas ^{153}Sm has higher energy ($E_{\beta,max}$ = 0.81MeV) and a longer particle range in tissues that allows for the deposition of high radiation doses in larger metastases. Furthermore, pain relief due to intravenous administration of ^{153}Sm-EDTMP, a beta-emitter with a physical half-life of 1.9 days, typically occurs within 1 week post injection [22], whereas it occurs within 2 week in the case of ^{177}Lu-EDTMP, a beta-emitter with a physical half-life of 6.7 days. Because of these complementary characteristics [23], we hypothesized that the combination of different radionuclides with different characteristics (radiation energies, half-life) such as ^{153}Sm and ^{177}Lu could be more advantageous to patients with painful bone metastasis.

To our knowledge, there is no study in the literature evaluating the use of the combined radiopharmaceutical of $^{153}Sm/^{177}Lu$-EDTMP for metastatic bone pain palliation therapy. This study aims to consider whether it is feasible to use combined radiopharmaceuticals for this purpose.

Therefore, the cocktail complex $^{153}Sm/^{177}Lu$-EDTMP was prepared. Contrary to our previous work [24], in this paper contribution of each radionuclide in the biodistribution of compositional radiopharmaceutical of $^{153}Sm/^{177}Lu$-EDTMP was separately evaluated. Also due to difference in radiolabeling of each component, radiolabeling yield of both components was separately measured by gamma spectroscopy method using HPGe detector. In order to find pharmacokinetics of $^{153}Sm/^{177}Lu$-EDTMP, based on its short (^{153}Sm) and long half-lives (^{177}Lu) components, sequential scintigraphic imaging was acquired at different time intervals (24 hours and 1, 2 week) after administration of the radiopharmaceutical.

METHODS

Preparation and quality control of $^{153}Sm/^{177}Lu$-EDTMP

$^{153}Sm/^{177}Lu$-EDTMP was prepared according to the previously described procedure [24]. In the first step, ^{177}Lu was produced by neutron irradiation of 150 µg of enriched Lu_2O_3 (^{177}Lu, 64.1% from Trace Inc.), according to the previous procedures at Tehran Research Reactor for a period of 7 days. Furthermore, ^{153}Sm was produced by neutron irradiation of 1 mg of enriched $^{152}Sm_2O_3$ (^{152}Sm, 98.7% from Trace Inc.) at a thermal neutron flux of 4×1013 n.cm-2.s-1 for 60 hours. The irradiated targets were dissolved in 1.0 M HCl to prepare 153Sm/ 177Lu chloride solution. In the second step, for labeling, an appropriate amount of the 153Sm/177Lu chloride solution containing the required activity was added to the desired amounts of EDTMP solution. The radiolabeling yield of the ligand was determined with paper chromatography

using Whatman No. 2 paper in NH4OH:MeOH:H2O (2:20:40) mixture.

Optimization study of $^{153}Sm/^{177}Lu$-EDTMP

To determine the effect of metal-to-ligand molar ratio (Me:EDTMP) on labeling yield, five vials containing Me:EDTMP molar ratio from 1:1 to 1:50 were used. The labeling yields of solutions were determined for various molar ratios. The solutions to be used in animals were first adjusted to pH 7 and were made sterile by using a Millipore filter prior to injection. Labeling yields of all the complexes under study were analyzed after changing Me:EDTMP molar ratio from 1:1 to 1:50. The radiolabeling yields of complexes were determined by paper chromatography. Five microliters of the test solution was spotted at 1 cm from bottom end of Whatman 3MM chromatography paper strips (10×1.5 cm). The strips were eluted with NH_4OH: methanol: water (0.2:2:4; v/v/v) mixture and then dried. Activity was measured using each 1 cm cut-sections of the strip separately in an HPGe detector. Labeled complex moved with the solvent front (Rf = 9–10), while ionic form remained at the point of spotting (Rf=0). Labeling yields of the complexes prepared at optimal parameters were also studied after 48 hours to evaluate the stability.

Biodistribution of $^{153}Sm/^{177}Lu$-EDTMP in rats

In order to determine the biodistribution of the radiolabeled complex in wild type rats, 0.1 ml of the $^{153}Sm/^{177}Lu$-EDTMP complex was injected intravenously into rats through their tail veins. The animals were sacrificed by CO_2 asphyxiation at the end of 2, 4, 24, 48 hours and 7 days post injection. The required tissues and the organs were excised for the calculations of percentage of activity per organ. The larger organs such as liver and intestines were weighed and then a small weighed portion was taken for the purpose of counting and adjusted for whole organ. Femur was taken as a representative for skeletal uptake. The associated activity was measured by a p-type HPGe detector using γ photons with energies of respective radionuclide.

Scintigraphic studies in rats

Scintigraphic studies were done on Wistar male rats to visually evaluate the distribution of EDTMP labeled with ^{177}Lu for comparison with ^{153}Sm. The Wistar rats were intravenously injected 0.15 ml of the complex solution containing ~150 µCi of each radionuclide through their tail vein. Planar images were acquired at the specified time intervals (24 hours and 1, 2 week after administration of the radiopharmaceutical) by a dual-head SPECT system with a low-energy-higher resolution (LEHR) collimator.

RESULTS AND DISCUSSION

Results of preparation and quality control of $^{153}Sm/^{177}Lu$-EDTMP

Specific activities of the produced ^{153}Sm and ^{177}Lu were 1.5 and 75 GBq/mg, respectively. In this work, the radiolabeling yield was calculated above 98% by using Whatman No. 2. The complex was found to be stable in final pharmaceutical sample.

The effect of molarities of EDTMP and radionuclide on labeling

Briefly, 0.1 ml of the complex solution containing ~100 µCi of ^{153}Sm and ~100 µCi of ^{177}Lu was added to five vials containing 0.1, 0.5, 1, 2, and 5 mg EDTMP which correspond to molar ratios (mole of Sm:mole of EDTMP) 1:1, 1:5, 1:10, 1:20 and 1:50, respectively. Radiolabelling yield of these formulations was determined to be 2.6%, 35.3%, 49.5%, 72.5% and 98.5%, respectively. Metal/ligand ratio versus percent labeling yields was depicted for $^{153}Sm/^{177}Lu$-EDTMP and each of its components separately in Figure 2. The graph clearly demonstrates 1:50 metal/ligand ratio is the optimum value in labeling process in vitro. It is worth mentioning, as well demonstrated in prior literature, in vivo stability (human clinical studies) requires a much higher ligand/metal ratio than that used in this study.

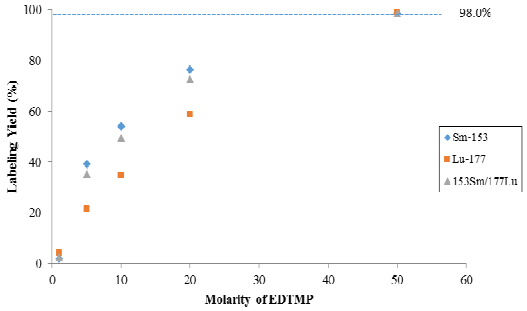

Fig 2. Labelling yield for various Metal:EDTMP molar ratios

Results of biodistribution of $^{153}Sm/^{177}Lu$-EDTMP in rats

$^{153}Sm/^{177}Lu$-EDTMP prepared at optimum labeling parameters and giving labeling yields of ~ 98% as mentioned in the above section were used for biodistribution studies in wistar rats up to 7 days post injection. Distribution of the activity in different organs (Figure 3) was calculated as the percentage of injected activity (dose) per g of organ (% ID/g).

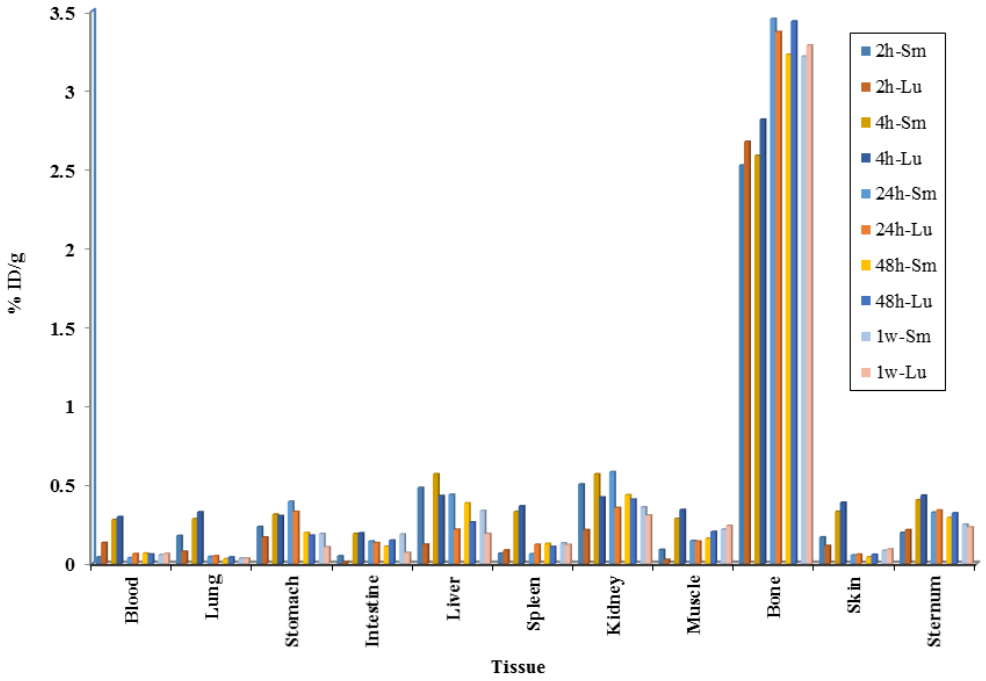

Fig 3. Biodistribution of $^{153}Sm/^{177}Lu$-EDTMP in wistar type rats (n = 3) based on measurement of ^{153}Sm and ^{177}Lu, separately.

The results are plotted in Figure 3 from which we can draw some general conclusions:

(i) The results of the biodistribution studies revealed significant bone uptake within 4 hours post injection. The observed uptake in femur was 2.5% and 2.7% ID/g at 4 hours post injection for ^{153}Sm and ^{177}Lu respectively. The femur uptake was observed to increase to 3.5% and 3.4% ID/g at 24 hours post injection.

(ii) Almost all the activity from the blood was cleared within 4 hours post injection.

(iii) Bone uptake remained almost constant until 1 week.

(iv) No significant accumulation of activity was observed in any of the major organs.

(v) Not only combined radiopharmaceutical is accumulated in target organ (bone) but also amount of distribution of its components and their quantities are similar. In other words, Results showed that both the radionuclides in the target organ are accumulated simultaneously and to the same extent.

Results of imaging Studies in Rats

The scintigraphic images of Wistar rats were recorded at 24 hours and 1, 2 week after the administration of $^{153}Sm/^{177}Lu$-EDTMP complex. In Figure 4A, the gamma camera was calibrated for 103 keV gamma photons of ^{153}Sm whiles in Figure 4B, the gamma camera was calibrated for 208 keV gamma photons of ^{177}Lu.

Some of the results that may be obtained include:

(i) The images (Figure 4) demonstrated selective uptake in the skeleton, findings which were in accordance with the biodistribution results.

(ii) At 24 hours post injection, the total skeleton was clearly visible and no uptake was observed in any other organ.

(iii) The skeletal activity was found to be retained without any significant leaching up to 2 week post injection.

(iv) The images showed the radionuclide with short half-life (high radiation rate) is almost decayed after two weeks, while the longer half-life radionuclide still is present in the target.

As a comparison, the labeling and QC of the $^{153}Sm/^{177}Lu$-EDTMP complex was more or less similar to both ^{153}Sm-EDTMP and ^{177}Lu-EDTMP.

Production of ^{153}Sm and ^{177}Lu with high radionuclide purity and sufficient specific activity makes them an desirable agent for therapeutic applications. The low energy β emission of ^{177}Lu can transfer high radiation doses to the small target. Because of its short emission range, most of its energy is deposited in

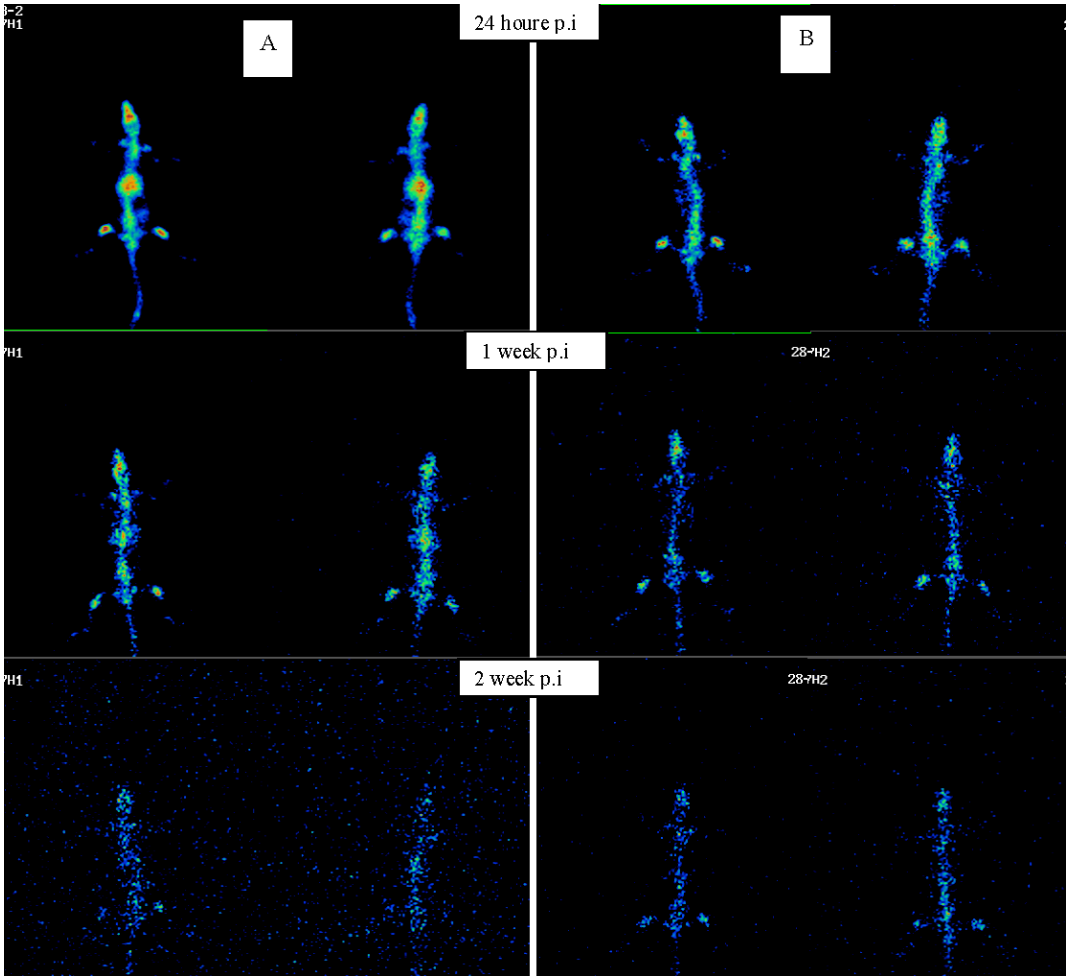

Fig 4. Scintigraphic images of $^{153}Sm/^{177}Lu$-EDTMP 24 hour and 1, 2 week post injection in wistar rat based on gamma photons: 103 keV for ^{153}Sm (A) and 208 keV for ^{177}Lu (B).

small metastases, whereas ^{153}Sm has higher energy and a longer particle range in tissues that allows for the deposition of high radiation doses in larger tumoral tissues. The high energy deposition of ^{153}Sm in larger tumoral tissues and the high deposition efficacy of ^{177}Lu in smaller tumors may result in desirable synergistic effects. Also the shorter half-life of ^{153}Sm leads to a higher dose rate and the longer half-life of ^{177}Lu leads to a lower and continuous dose rate. Because of these supplementary specifications, the combination of ^{153}Sm and ^{177}Lu would be quite reasonable. The results show that ^{153}Sm and ^{177}Lu could be simultaneously labeled with EDTMP with radiochemical purity of 98%. Also, the preparation of $^{153}Sm/^{177}Lu$-EDTMP radiopharmaceutical is simple and the complex is stable.

$^{153}Sm/^{177}Lu$-EDTMP radiopharmaceutical was administered intravenously through the tail vein of wistar rats and biodistribution data were collected from 2 hours to 7 day post injection. Biodistribution studies of $^{153}Sm/^{177}Lu$-EDTMP revealed high skeletal uptake (3.5% and 3.4% ID/g at 24 hours post injection for ^{153}Sm and ^{177}Lu respectively) with rapid blood clearance and minimal uptake in any of the major organs. Uptake of both the radionuclides in the bone occurred simultaneously with similar extent.

Scintigraphic images of the wistar rats injected with $^{153}Sm/^{177}Lu$-EDTMP at 24 hours and 1, 2 week after administration of radiopharmaceutical, verified high skeletal uptake. The skeleton was clearly visible, and no significant uptake in other organs was observed.

CONCLUSION

Our study indicates that using combined radiopharmaceutical is feasible and safe. Furthermore, $^{153}Sm/^{177}Lu$-EDTMP could be specially advantageous when tumoral lesions of different sizes are present.

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