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Original Article

Prediction of radiation dose to adult human from radiopharmaceutical manufactured by third generation bisphosphonate labeled with Rhenium

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ABSTRACT

Introduction: The crucial step in preclinical process of radiopharmaceutical production is internal dosimetry evaluation by different ways to realize radiobiological dose-response relationships and to extract the results for clinical use. Till now several bone-seeking radiopharmaceuticals have been developed for bone metastasis. Interesting features of bisphosphonates attracted attentions to them in the field of radiopharmaceutical therapy and studies on new generation of them have been doing too.
Materials and methods: In this study, we used ZNA as representative of the third generation. The radiopharmaceutical ^{188}Re-ZNA was produced and its radiochemical purity was investigated. Then, the biological distribution of the produced radiopharmaceutical at 1, 2, 4 and 24 h after injection on different organs of mice were investigated. Finally, the absorbed dose of organs in the human body was assessed using the RADAR method.
Results: The results show 96% radiochemical purity of the ^{188}Re-ZNA radiopharmaceutical. The amount of %ID/g in bone is 1.131% after 1 h and in 24 h it has a significant amount compared to other organs, that is 0.516%. Also dosimetric results show that the highest absorption dose is related to bone and the amount of this dose is 0.050 mGy/MBq.
Conclusion: Considering the possibility of producing the ^{188}Re-ZNA radiopharmaceutical, as well as the proper distribution of this radiopharmaceutical in target and non-target organs and increasing the absorbed dose in bone, it can be concluded that this radiopharmaceutical can be useful in the "radiopharmaceutical therapy" in metastases.

1. Introduction

To assessment new radiopharmaceuticals for therapeutic or diagnostic purposes in nuclear medicine the absorbed radiation dose from internally deposited radionuclides is the most important factor in estimating risks or therapeutic efficacy. Radiopharmaceutical dosimetry process by illustrating an overview of the energy accumulation associated with the emission of radiopharmaceuticals and the patient's body, provides the optimal clinical use of radiopharmaceuticals. The new radiopharmaceuticals establishment is based on Radiation dosimetry during their development. The absorbed dose, is an estimated amount which is calculated based on considering localized uptake and retention of administered radiopharmaceutical, the used radionuclide radiation decay data and simulation of the radiation transport to human body models [1].

In therapeutic applications, there is possible concern that large amount of radiation dose, is received by other organs especially by radiosensitive organs. In spite of the fact that it is preferable to measure directly the absorbed dose and the dose distribution in the body, it is not usually possible for routine clinical investigations [2]. Although in vitro models are available to evaluate some of the factors of the safety profile of radiopharmaceuticals, they are clearly not sufficient to adequately predict the biodistribution and dosimetry of radiopharmaceuticals after injecting to humans. The use of preclinical experiments is more developed and helpful than in vitro measurements, because its results is close to the response of humans [3]. So one of the preliminary steps in assessments of

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new radiopharmaceuticals before performing human measurements, is dosimetry studies using non-human biological systems.

This procedure is useful for subsequent clinical applications in humans and can accelerate future investigations. This method is based on similar biological distributions of activity between animals like rats and human tissues, organs, and excretory pathways. Using preclinical studies and animal data for absorbed dose estimation in humans presents new paths to the application of radioactive compounds in clinical trials [4,5].

Bisphosphonates (BPs) based on structural differences from R1 side chain can be classified into three generations. The first generation of BP (e.g., clodronate and etidronate) does not contain nitrogen while the second generation of BP (e.g. pamidronate and alendronate) in the R1 side chain contains nitrogen. The third generation of BP (e.g., ibandronate and zoledronate) includes nitrogen in circular shape structure.

Among them the third generation bisphosphonates which has heterocyclic nitrogen containing side chain have a high affinity for hydroxyapatite crystals in bone and is powerful antiresorptive agent. In preclinical models of bone resorption, the zoledronic acid is the maximum powerful examined bisphosphonate. For example, zoledronic acid is at least 100 times more effective than pamidronate or clodronate in preclinical models of bone resorption, and at least 1000 times more powerful than etidronate [6]. Zoledronic acid is an imidazole compound having a 2,2-bis(phosphono)-2-hydroxyethane-1-yl substituent at the 1-position. It has a role as a bone density conservation agent. It is a member of imidazoles and a 1,1-bis(phosphonic acid) [7]. This highly potent and new-generation bisphosphonate is the first bisphosphonate to demonstrate efficacy in patients with bone metastases from solid tumors other than breast cancer, including prostate cancer, non–small-cell lung cancer, and many of other tumor kinds [8]. Zoledronic acid chemical structure is shown in Fig. 1.

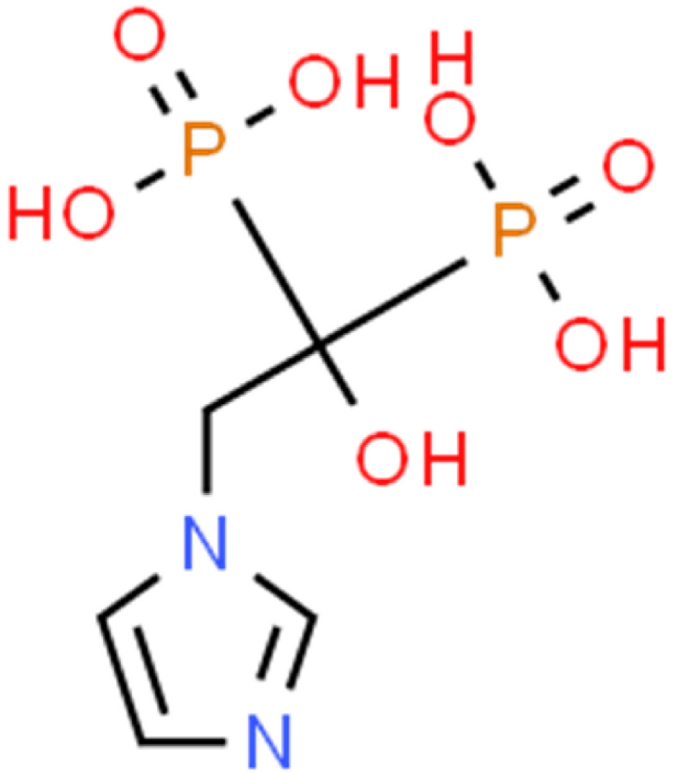

Fig. 1. Chemical structure of zoledronic acid.

In this research the ^{188}Re-ZNA has been produced and the authors have tried to estimate the absorbed radiation dose to human organs following intravenous administration of produced radiopharmaceutical. To evaluate absorbed radiation dose, the radiation dose assessment resource (RADAR) method and OLINDA/EXM software which is based on the stylized anatomical models developed for the MIRD Committee of The Society of Nuclear Medicine in the 1960s [6] have been used as a common procedure in nuclear medicine.

2. Material and methods

$^{188}ReO_4^-$ was produced by $^{188}W/^{188}Re$ (Pars Isotope, Iran) radionuclide generator with tungsten-188 loaded on an alumina (Al_2O_3) absorbent eluted with saline solution (0.9% NaCl). ZNA kit was prepared from Pars Isotope Company. Other Chemical agents were prepared from Sigma/Aldrich.

The activity of $^{188}ReO_4^-$ was measured by a dose calibrator (Isomed, Germany). An EG&G/ORTEC model 4001 M Mini Bin & Power Supply NaI(Tl) counter was used for gamma counting. Radiochromatography was performed by counting of Whatman No. 1 using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Animal experiments were performed in accordance to the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) and comply with the U.K. Animals Act, 1986.

2.1. ^{188}Re-ZNA Preparation and quality control

To produce ^{188}Re-ZNA the first step is preparation the relevant radionuclide. So to achieve ^{188}Re, the radionuclide generator $^{188}W/^{188}Re$ (Pars Isotope, Iran) was used. ZNA kit was prepared from Pars Isotope Company. Next, ^{188}Re was added to the kit. Then, the pH of the solution was adjusted by adding HCl (optimum pH of the solution between 1 and 3). After that, the prepared mixture was stirred for 30 s, and then the vials were placed in boiling water for 30 min. Finally, the composition was cooled and reached the room temperature.

The yield of the radiolabeling was evaluated by spotting 5 μL of samples on Whatman No 1 as stationary phase. Chromatography paper was used for radiochemical purity monitoring. These stripes were then developed in saline and acetone solutions as mobile phases until the solvents reached the top of the strips. After that, a thin layer chromatography scanner, Bioscan AR2000, Paris, France, used the chromatography system to analyze TLC.

In the using of acetone as mobile phase, $^{188}ReO_4^-$ moves up with the solvent, while colloidal impurities $^{188}ReO_2$ and ^{188}Re-ZNA both remained at the starting point. In using saline at mobile phase $^{188}ReO_4^-$ and ^{188}Re-ZNA move upwards with the solvent and the colloidal impurity $^{188}ReO_2$ remains at the spotting point. The TLC analysis was then performed by a chromatographic system which mentioned above.

2.2. Biodistribution studies

Animal experiments were performed in accordance to the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) and comply with the U.K. Animals Act, 1986. To determine biodistribution, ^{188}Re-ZNA was administered to mice (weights: 27–32 gr). Three mice at each time intervals of 1, 2, 4, 24 h were sacrificed after injection of 0.540 ± 0.015 mCi activity of produced ^{188}Re-ZNA. The activity of different vital organs was calculated as a percentage of the injected dose per gram using a NaI (Tl) detector (% ID/g).

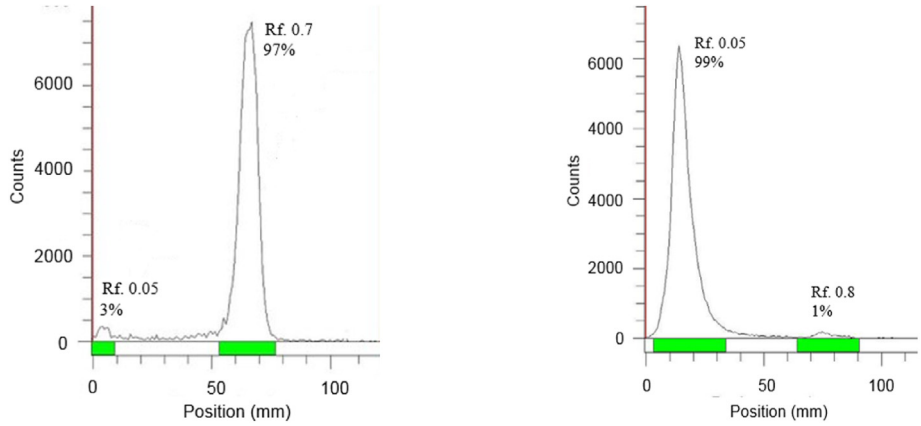

Fig. 2. TLC chromatograms of ^{188}Re-ZNA with Whatman No. 1 paper in saline (left) and acetone (right).

$$\%ID/g(t) = \frac{A_{Organ}(t)/M_{Organ}}{A_{Total}(t)} \quad (1)$$

Where $A_{Organ}(t)$ is the ^{188}Re-ZNA activity in the sample, M_{Organ} is the mass of the sample and $A_{Total}(t)$ is the total activity of produced radiopharmaceutical injected to the mice. To calculate the time-integrated activity which is shown by $\tilde{A}$ in source organs, all the measured activities were decay corrected to the time of sacrificing, the clearance curve for each organ was plotted and the area under the curves was computed.

2.3. Dosimetric evaluation

A plot of the distribution data for each organ was generated and the accumulated activity was calculated by computing the area under the curves. The decay-corrected time-activity curves were extrapolated to infinity by fitting the tail of each curve to mono-exponential curve and integrated, resulting in cumulated activity $\tilde{A}$ for each organ.

After computing the area under the curve which resulted in accumulated activities, this calculated accumulated activities were extrapolated to human body by the proposed method of Sparks and Aydogan [9,10].

$$\tilde{A}_{HO} = \tilde{A}_{AO}\,\frac{Mass_{HO}/Mass_{HB}}{Mass_{AO}/Mass_{AB}} \quad (2)$$

Which in the above formula HO indicates Human Organ, AO indicates Animal Organ, HB indicates Human Body, AB indicates Animal Body.

2.4. Absorbed dose calculations

The absorbed dose in human organs was calculated by using RADAR method which formulated by Stabin and Siegel [10]:

$$D = N \times DF \quad (3)$$

Where N is the number of disintegrations that occur in a source organ and DF (in mGy/MBq s) is the Dose Factor.

DF shows the physical decay features of the radionuclide, the range of emitted radiations and the size of organ and configuration and is defined as:

$$DF = \frac{k\sum_i n_i E_i \varnothing_i}{m} \quad (4)$$

Where the number of radiations with energy E emitted per nuclear transition was shown with n_i, the energy per radiation (MeV) is E_i, the fraction of energy emitted in source that is absorbed in the target is $\varnothing_i$, the mass of target region (kg) is m and k is some proportionality constant (mGy kg/MBq s MeV). In this study DFs have been taken from the OLINDA/EXM software [11].

3. Results and discussion

^{188}Re was prepared from ^{188}W generator. No other gamma-emitting radionuclide was detected by gamma spectrometry in all productions. Zolendronic acid was radiolabeled with the prepared ^{188}Reradionuclide at the optimized condition. The labeling process of ZNA with ^{188}Re was done at the next step.

In chromatography each component in a given sample is characterized by Rf (Retention factor) value, which is defined as the ratio of the distance passed by the considering component to the distance that solvent front has advanced from the original point of application of the test material. These values are established with known components and may change under different experimental situations. The Rf values are used for the identification of different components in a sample [12]. The green part in Fig. 2 is the part of Whatman paper where the count of gamma rays emitted from rhenium is recorded.

After radiopharmaceutical injection, the animals were sacrificed by CO_2 asphyxiation at selected time points (1, 2, 4, 24 h). Dissection started by extracting blood from the aorta and samples of the vital organs like bone, kidneys, heart, spleen and etc., were removed. The tissue uptakes was calculated based on the measurements of NaI(Tl) detector. The results are shown in Table 1.

For better evaluation the time–activity curves for crucial organs are shown in Fig. 3. %ID/g is the radioactivity of different organs that was calculated as a percentage of the injected dose per gram of organ.

RADAR method and the biodistribution data in mice organs was used to evaluate the dosimetric assessment in human organs. Table 2 presents the subsequent absorbed dose in each human organ after injection of produced radiopharmaceutical.

In testing new radiopharmaceuticals, the first step before starting human studies is the dosimetric researches based on in vivo animal biokinetics. The animal studies can be useful to human clinical purposes and hasten later investigations. This

Table 1
^{188}Re-ZNA Biokinetics in different organs of mice.

Tissues	%**ID**/**g**			
	1 h	2 h	4 h	24 h
Blood	0.331 ± 0.030	0.201 ± 0.016	0.089 ± 0.005	0.030 ± 0.002
Bone	1.131 ± 0.102	0.97 ± 0.078	0.812 ± 0.049	0.516 ± 0.026
Muscle	0.027 ± 0.002	0.019 ± 0.002	0.017 ± 0.001	0.008 ± 0.001
Stomach	0.378 ± 0.034	0.298 ± 0.024	0.167 ± 0.01	0.012 ± 0.001
Heart	0.095 ± 0.01	0.060 ± 0.005	0.022 ± 0.001	0.007 ± 0.001
Lung	0.98 ± 0.088	0.814 ± 0.065	0.189 ± 0.011	0.087 ± 0.004
Intestine	0.782 ± 0.07	0.648 ± 0.052	0.532 ± 0.032	0.089 ± 0.004
Thyroid	0.320 ± 0.029	0.291 ± 0.023	0.167 ± 0.01	0.072 ± 0.004
Spleen	0.980 ± 0.09	0.079 ± 0.006	0.046 ± 0.004	0.031 ± 0.002
Kidney	4.191 ± 0.38	3.010 ± 0.24	1.280 ± 0.08	0.320 ± 0.017
Liver	1.451 ± 0.13	1.369 ± 0.11	0.739 ± 0.044	0.138 ± 0.008

strategy is based on that truth which the activity biodistribution among different tissues, organs and excretory routes in investigational animals and human are comparative [4].

Dosimetry studies based on in vivo animal biokinetics are essential to keep the radiation dose in humans as low as possible. Preclinical experiments like this study, can open new paths for radiopharmaceutical use in the future [13].

Animal biodistribution data was used to ^{188}Re-ZNA evaluation up to 24 h post injection. After producing ^{188}Re-ZNA and performing subsequenting processes like quality control, the ^{188}Re-ZNA was injected to mice and the animals were sacrificed at special times. The activities of different organs were measured.

Table 1 shows that the radiopharmaceutical was cleared quickly from the blood (%ID/g = 0.331% at 1 h and 0.030% at 24 h), which proves that this organic compound has a short physiological half-life.

Noticeable amount of activity is accumulated in the bone as expected. %ID/g in bone showed 1.131% after 1 h and 0.97% after 4 h. As it can be seen from Table 1, however the bone %ID/g amount decreases with time, but its declining trend is slow in comparison to another organs and at the end of study time, half of the %ID/g amount of the bones (0.516%) still remains in them compared to the initial time of the test (1.131%). These values indicate the high persistence of the radiopharmaceutical in the target organ, that is, the bone.

Table 2
The absorbed dose in each human organ after injection of^{188}Re-ZNA.

Target organ	Absorbed dose (mGy/MBq)
Breasts	0.000
SI	0.017
Stomach wall	0.001
ULI wall	0.000
Heart wall	0.001
Kidneys	0.021
Liver	0.021
Lungs	0.005
Muscle	0.001
Ovaries	0.000
Red marrow	0.028
Bone	**0.050**
Skin	0.000
Spleen	0.003
Testes	0.000
Thyroid	0.005
Total body	0.004

Abbreviations: SI: small intestine; ULI: upper large intestine.

After ^{188}Re-ZNA injection, the %ID/g of radiopharmaceutical by the kidney after 1 h was 4.191%, which decreased to 1.280% in 4 h. This process of absorption and excretion indicates that the main route of elimination of the produced radiopharmaceutical is renal excretion.

The amount of ID/g % was taken by the liver after 1 h is 1.451%, which after 4 h reaches to 0.739% and in 24 h it reaches to small amount of 0.138%. As a result, in addition to renal excretion, which is the main excretion pathway, the produced radiopharmaceutical also has hepatic excretion. So this high %ID/g amounts don't cause any concern, as like kidneys.

The time–activity curve for each organ was plotted and the accumulated activity value of mice was calculated by computing the area under the organ curves. The amount of the radiation-

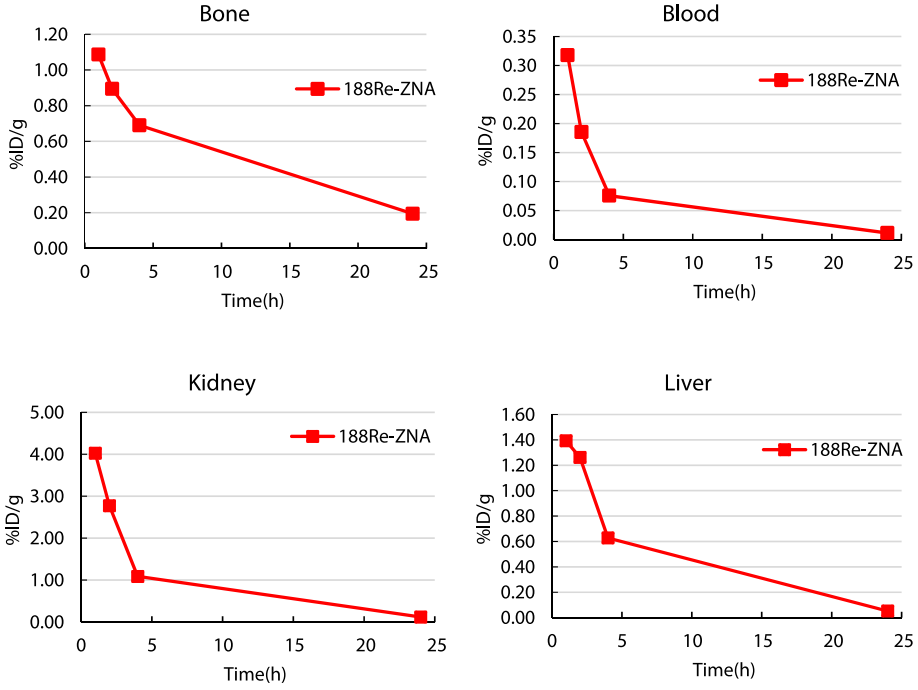

Fig. 3. The decay-corrected time-activity curves.

absorbed dose to a human was calculated by multiplying the converted mice's activities (using relative organ mass extrapolation) to the DF values of ^{188}Re have been taken from the OLINDA/EXM software. The calculated absorbed dose of various organs and tissues are presented in Table 2.

As Table 2 shows the highest amount of calculated human absorbed dose of produced radiopharmaceutical is in bone with amount of 0.050 mGy MBq^{-1}. After bone, the higher amounts are seen in bone marrow (0.028 mGy MBq^{-1}), liver and kidney (0.021 mGy MBq^{-1}). These amounts show that these organs dose amounts are too low in comparison with bone.

4. Conclusions

Using non-human biological systems for dosimetry studies to determine the safety and efficacy of radiopharmaceuticals before they are injected to the patients is important step in preclinical phase. Bisphosphonates are used in treatment of specific bone diseases, such as multiple myeloma and osteo-high metastatic cancers. Since these compounds stimulate bone repair, their function in the treatment of painful metastatic bone lesions is growing.

In this regard in our study, the biodistribution of ^{188}Re-ZNA was investigated in different time points in the mice. Subsequently, the human absorbed dose of the produced radiopharmaceutical was estimated based on biodistribution data in mice. Based on our measurements and calculations the dose of different organs of the human body, the results showed that the absorbed dose of ^{188}Re-ZNA in the target tissue (bone) is much higher than other tissues. This value in bone and bone marrow is equal to 0.050 and 0.028 mGy/MBq^{-1}, respectively.

According to the possibility of producing the ^{188}Re-ZNA radiopharmaceutical and also the proper distribution of this radiopharmaceutical in target and non-target organs and also high amount of absorbed dose in bone, it can be said that ^{188}Re-ZNA can be helpful in the “radiopharmaceutical therapy” for bone metastases.

Stability and high bone absorption are important features of this radiopharmaceutical. Based on the observed characteristics, with further studies, this radiopharmaceutical can be used to relieve bone pain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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