

Human organ absorbed dose estimation of ^{166}Ho -BPAMD complex based on biodistribution data of male Syrian rats

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ABSTRACT

Introduction: Recently, ^{166}Ho -BPAMD was introduced as a suitable agent for bone marrow ablation. The aim of this study was to estimate the absorbed dose of this novel agent in the human organs which is necessary before the clinical application.

Methods: ^{166}Ho was produced by direct irradiation of ^{165}Ho in the research reactor. 250 μg of BPAMD was added to the vial containing 111 MBq of ^{166}Ho and the pH of the reaction mixture was adjusted to 6 while it was incubated for 45 min at 90-100°C. The strong cation exchanger was applied to improve the radiochemical purity checked by ITLC method. ^{166}Ho -BPAMD was injected to male Syrian rats and the uptake in different organs was assessed. The absorbed dose in human organs was estimated following the mass extrapolation and according to RADAR method.

Results: ^{166}Ho -BPAMD was prepared with the radiochemical purity of higher than 96%. After injection to male Syrian rats, the most of the activity was observed in the bone tissues. Bone surface and bone marrow received the highest amounts of the absorbed dose with the value of 0.916 and 0.647 mGy/MBq, respectively.

Conclusion: Bone marrow to the bone tissue and total body absorbed dose ratio for ^{166}Ho -BPAMD was comparable to the other bone seeking radiopharmaceuticals. ^{166}Ho -BPAMD delivers safe and reasonably appropriate dose to the human organs and can be considered as a novel bone marrow ablative agent.

Key words: Absorbed dose; Bone marrow ablation; BPAMD; ^{166}Ho ; Syrian rats

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INTRODUCTION

Recently, numerous bone-seeking radiopharmaceuticals have been developed for bone marrow ablation in patients with hematological malignancies including multiple myeloma [1-4]. Radionuclides with higher beta particle energies such as ¹⁵³Sm, ¹⁶⁶Ho and ⁹⁰Y are expected to have greater efficacy for bone marrow ablation [5-6]. In view of high tendency of bisphosphonates for accumulation in bone, various radiopharmaceuticals have been introduced containing phosphonate ligands to deliver high levels of radiation to bone and bone marrow while sparing normal tissues [7].

¹⁶⁶Ho-DOTMP is the most well-known radiopharmaceutical for bone marrow ablation. While ¹⁵³Sm-EDTMP is introduced as an agent for pain relief of bone metastases [8]. High dose ¹⁵³Sm-EDTMP showed encouraging results in total marrow irradiation [9]. Following more researches to obtain ligands with better characteristics, The (4{[(bis(phosphonomethyl)carbamoyl)methyl]-7,10-bis (carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl}acetic acid (BPAMD) was presented demonstrating high affinity to bone tissue with various diagnostic and therapeutic radionuclides labeled, resolving some of the restriction of the commonly used phosphonates like low in-vivo stability [10-12].

Production of ¹⁶⁶Ho-BPAMD with radiochemical purity of higher than 94% is recently reported showing significant stability at room temperature and in human serum for at least one half-life [13]. Biodistribution and imaging studies of this radiolabelled compound in normal Syrian mice demonstrated the localization of the major portion of the injected activity from blood circulation into bones. According to the results, the authors believe that this agent has interesting characteristics for bone marrow ablation. Nevertheless, more research specially biological and dosimetric investigations are needed before clinical application.

One of the most important parameters that should be undertaken in the administration of the radiopharmaceuticals is the radiation absorbed dose received by the target and non-target organs. Therefore, the knowledge of the absorbed dose by various critical organs is an essential step in developing any radiopharmaceutical specially in therapy applications [14-15].

Estimation of the absorbed dose to organs is a prerequisite for the clinical application of a new radiopharmaceutical and can be obtained translating biodistribution data in small animals to humans [16]. In fact, prior to moving forward with human measurements from a small number of volunteers, consistent with the recommendations of ICRP 62 [17]. This estimation is a common step to accelerate the development of radioactive compounds in clinical

applications. Nowadays, the most commonly used procedure for making the internal absorbed dose estimates is the radiation absorbed dose assessment resource (RADAR) method [18].

In this study, the human organ absorbed dose after injection of ¹⁶⁶Ho-BPAMD was estimated based on biodistribution data in wild-type rats. Relative organ mass extrapolation was used for the determination of accumulated activity in human organs. Calculation of absorbed dose was carried out according to RADAR method. Eventually, bone marrow to bone tissue and total body absorbed dose ratios of ¹⁶⁶Ho-BPAMD was compared with values for some other common bone-seeking radiopharmaceuticals.

METHODS

Natural holmium nitrate with purity of >99.99% and BPAMD was prepared from ISOTEC Inc and ABX (Radeberg, Germany), respectively. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Whatman No. 2 paper was obtained from Whatman (Buckinghamshire, U.K.). Gamma impurities and beta impurities were determined using an high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-500SL) multichannel analyzer and a Wallac 1220 Quantulus, Perkin Elmer, Ultra low-level liquid scintillation spectrometer (Turku, Finland). A bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA) was used for reading of TLC-chromatography papers. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines [19]. All values were expressed as mean ± standard deviation (Mean ± SD) and the data were compared using student T-test. Statistical significance was defined as P<0.05.

Preparation and quality control of ¹⁶⁶Ho-BPAMD

¹⁶⁶Ho was produced by neutron irradiation of natural ¹⁶⁵Ho(NO₃)₃ (¹⁶⁵Ho, 99.99% from ISOTEC Inc.) via ¹⁶⁵Ho(n, γ) ¹⁶⁶Ho reaction and in accordance with the reported procedures [20]. BPAMD was radiolabeled with ¹⁶⁶Ho according to the previously reported literature [13] whereas its radiochemical purity was determined using ITLC method and NH₄OH:MeOH:H₂O (0.2:2:4) mixture as the mobile phase.

Biodistribution of ¹⁶⁶Ho-BPAMD in male Syrian rats

For cumulated activity evaluation in the target and non-target organs, male Syrian rats weighing 180-220 g kept at routine day/night light program and under common rodent diet pellets were scrutinized. 100 μL of the final radiolabeled complex was injected intravenously into the rats through their tail veins.

The animals were sacrificed at selected times after injection under the animal care protocols. The tissues were rinsed with normal saline, the weight was determined with a calibrated balance and the activity of each organ was determined with a p-type coaxial HPGe detector and a Quantulus counter. Eventually, the non-decay corrected percentage of the injected dose per gram (%ID/g) for different organs was calculated.

Statistical analysis

Five rats were sacrificed for each time interval. All values were expressed as mean ± standard deviation and the data were compared using Student's T-test. The statistical significance was defined as P<0.05.

Calculation of cumulated activity in human organs

The cumulated source activity for each animal organ was calculated according to Equation 1, where A(t) is the activity of each organ at time t and t₁ is the first injection time.

$$\tilde{A} = \int_{t_1}^{\infty} A(t) dt \tag{1}$$

To calculate the cumulated activity in the animal organs (according to Eq. 1), the authors used the trapezoidal method up to the last time point, and then calculated the remaining area analytically, assuming the radioactive decay from the last time point to infinity. Therefore, the non-decay corrected percentage-injected activity versus time was plotted for each animal organ. Linear approximation was used between the two experimental points of time. The curves were extrapolated to infinity by fitting the tail of each curve to a monoexponential curve with the exponential coefficient equal to the physical decay constant of each radionuclide. Whereas, the activity of blood at t=0 was considered as the total amount of the injected activity, the activity of all other organs was assumed to be zero at that time.

The cumulated activity for animal organs was then extrapolated to the cumulated activity for human organs by the proposed method of Sparks et al. (Eq. 2) [21].

$$\%A_{\text{human organ}} = \%A_{\text{animal organ}} \times \frac{\text{OrganMass}_{\text{human}} / \text{BodyMass}_{\text{human}}}{\text{OrganMass}_{\text{animal}} / \text{BodyMass}_{\text{animal}}} \tag{2}$$

Equivalent absorbed dose calculation

The absorbed dose in human organs was calculated by RADAR formalism based on biodistribution data in the rats [18] according to the other previously reported research [22]. Briefly, the calculated cumulated activity for each source organ was multiplied by the

dose factors (DFs) for the related organ. The total absorbed dose for each target organ was computed by the summation of the absorbed dose delivered from each source organ. For this research, DFs have been derived from the data reported in OLINDA/EXM software [23].

RESULTS

Preparation and quality control of ¹⁶⁶Ho-BPAMD complex

¹⁶⁶Ho was prepared with radionuclidic and radiochemical purity of higher than 99%. The radiolabeling yield of higher than 96% was achieved for ¹⁶⁶Ho-BPAMD preparation. Utilizing Whatman No.2 papers and NH₄OH:MeOH:H₂O (0.2:2:4) solvent system, the radiolabeled compound migrated to the higher R_f (0.7-0.8), whereas the free cation remained at its origin.

Biodistribution of ¹⁶⁶Ho-BPAMD in male Syrian rats

Biodistribution of the radiolabeled complex in male Syrian rats was studied up to 72 h post injection. The non-decay corrected %ID/g for the rat organs after the injection of the complex are expressed as mean ± standard deviation and presented in Table 1.

Dosimetric studies

Dosimetric evaluation of the complex was carried out by the RADAR method. The equivalent absorbed dose values extrapolated in human organs after intravenous injection of the complex is presented in Table 2.

DISCUSSION

In this study we aimed to estimate human absorbed dose of ¹⁶⁶Ho-BPAMD which is crucial before clinical application of this novel bone-marrow ablative agent. The biodistribution data in male Syrian rats and the previously reported methodology were utilized [12, 22]. Despite the observed differences between the doses from human data and those obtained from studies of rodents, previous studies have demonstrated the usefulness of using animal distribution as a model for absorbed dose estimations in humans [24].

The biodistribution data showed fast removal of ¹⁶⁶Ho-BPAMD from blood circulation and high accumulation in bone tissue which is in accordance with expectations from bone-seeking radiopharmaceuticals as previously reported [13]. However, the major route of excretion for the labelled compound is through the urinary tract, the significant excretion of the radioactivity is observed by the kidneys as anticipated due to the water solubility of the compound.

Table 1: The non-decay corrected %ID/g values after intravenously injection of ¹⁶⁶Ho-BPAMD to the rats.

Organ	Time				
	2 h	4 h	24 h	48 h	72h
Blood	0.07 ± 0.01	0.07 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bladder	0.65 ± 0.03	0.38 ± 0.02	0.14 ± 0.01	0.06 ± 0.01	0.01 ± 0.00
Kidney	1.09 ± 0.05	0.50 ± 0.03	0.11 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Liver	0.25 ± 0.01	0.39 ± 0.01	0.10 ± 0.00	0.03 ± 0.00	0.00 ± 0.00
Spleen	0.06 ± 0.00	0.14 ± 0.01	0.02 ± 0.00	0.05 ± 0.00	0.00 ± 0.00
Lung	0.02 ± 0.00	0.21 ± 0.01	0.31 ± 0.01	0.10 ± 0.00	0.05 ± 0.00
Stomach	0.06 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Heart	0.01 ± 0.00	0.15 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00
Intestine	0.05 ± 0.00	0.09 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Bone	7.42 ± 0.31	7.14 ± 0.38	4.06 ± 0.30	1.98 ± 0.23	0.89 ± 0.18
Muscle	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.01 ± 0.00
Skin	0.00 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

The highest amounts of the absorbed dose after the injection of ¹⁶⁶Ho-BPAMD was observed on the bone surface (0.916 mGy/MBq) and in the bone marrow (0.647 mGy/MBq). The other organs would receive insignificant dose which is the main advantage of this compound.

Table 2: Equivalent absorbed dose delivered into human organs after injection of ¹⁶⁶Ho-BPAMD.

Target Organs	¹⁶⁶ Ho-BPAMD (mGy/MBq)
Small Intestine	0.004
Stomach Wall	0.002
Heart Wall	0.008
Kidneys	0.100
Liver	0.050
Lungs	0.112
Muscle	0.080
Red Marrow	0.647
Bone Surface	0.916
Spleen	0.111
Total Body	0.102

The most important goal in developing therapeutic radiopharmaceuticals is to deliver the maximum possible absorbed dose to the target organ and the minimum possible absorbed dose to the non-target organs. Therefore, the target to non-target absorbed dose ratios are important parameters to be considered. The bone marrow to the bone surface and total body absorbed dose ratios after injection of ¹⁶⁶Ho-BPAMD complex are given in Table 3. For better comparison,

these ratios reported for the other bone seeking agents are also presented in this table [5, 12].

Table 3: Bone marrow to non-target absorbed dose ratios for ¹⁶⁶Ho-BPAMD and other bone seeking radiopharmaceuticals [5, 12].

Organs	Bone Surface	Total Body	Reference
¹⁶⁶ Ho-BPAMD	0.7	6.6	This study
¹⁶⁶ Ho-PAM	0.8	9.5	[5]
¹⁶⁶ Ho-TTHMP	0.8	9.3	[5]
¹⁵³ Sm-BPAMD	0.2	3.7	[12]
¹⁵³ Sm-EDTMP	0.2	4.2	[12]

Since estimation of the absorbed dose of human organs from the biodistribution data in rats may have some over- or underestimation we tried to compare the data with the absorbed dose estimation of other bone seeking radiopharmaceuticals based on the biodistribution data in rats.

As can be seen in Table 3, the red marrow to bone surface and total body absorbed dose ratios for ¹⁶⁶Ho-BPAMD are higher compared to ¹⁵³Sm agents (¹⁵³Sm-EDTMP and ¹⁵³Sm-BPAMD) demonstrating the superiority of this new radiolabelled compound. However, these ratios for ¹⁶⁶Ho-PAM and ¹⁶⁶Ho-TTHMP are a bit higher, according to this data, ¹⁶⁶Ho-BPAMD appears to most adequate and safe dose for therapeutic applications.

CONCLUSION

The ¹⁶⁶Ho-BPAMD complex was prepared with radiochemical purity of higher than 96%. In the

normal Syrian rats, the biodistribution of the complex indicated the highest uptake in the bones. Bone surface and bone marrow showed the highest amounts of the absorbed dose following injection of ^{166}Ho -BPAMD with the values of 0.916 and 0.647 mGy/MBq, respectively. The absorbed dose of the other organs were insignificant. Bone marrow to the bone tissue and total body absorbed dose ratios for ^{166}Ho -BPAMD was higher than these ratios for ^{153}Sm agents which was expected due to the higher energy of the beta particles comparable to the ratios for the other ^{166}Ho complexes. Generally, this novel agent can deliver high and safe dose to bone marrow considered as to be a good candidate for ablation. However, for a better evaluation of the efficacy of this novel agent, more studies in animal models with bone marrow malignancies specially multiple myeloma are warranted.

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