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Absorbed Dose of Human Organs after Injection of ^{68}Ga-EDTMP: Estimation from Biodistribution Data in Wild-Type Rats

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Abstract

Purpose: ^{68}Ga is a generator-based radionuclide with suitable characteristics for PET imaging. ^{68}Ga-EDTMP has recently been introduced as a new agent for bone imaging. In this study, the human absorbed dose of this agent was calculated according to RADAR method and based on the bio-distribution data in Wild-type rats. **Materials and Methods:** ^{68}Ga was obtained from ^{68}Ge/^{68}Ga generator. While, the radiolabeled complex was prepared in the optimized conditions, the radiochemical purity was checked by Instant Thin Layer Chromatography (ITLC) method. Absorbed dose of each human organ was calculated following the bio-distribution assessment of the complex in the Wild-type rats up to 120 min.

Results: ^{68}Ga was prepared with radionuclidic purity and radiochemical purity of higher than 99%. The results indicated the radiochemical purity of higher than 99% for ^{68}Ga-EDTMP. As expected, bone surface and bone marrow with 0.112 and 0.053 mSv/MBq, respectively, received the highest absorbed dose. The dose of total body was estimated to be 0.006 mSv/MBq.

Conclusion: according to the results, the radiolabeled complex can be considered as a safe agent for bone imaging.

1. Introduction

Skeletal metastasis is a common cause of severe morbidity [1] which accounts for 70% of all malignant bone tumors [2]. While the vast majority of skeletal cancers are originated from the other cancers rather than primary bone tumors [3], lung cancer, breast cancer, renal cell carcinoma and prostate cancer include approximately 80% of all skeletal metastases [2].

Skeletal metastases can result in severe pain, hypercalcemia, pathologic fracture and spinal cord compression [4, 5]. The quality of life in patients suffering from this abnormality reduced substantially. Despite of the early occurrence of skeletal metastases in the tumor disease, their symptoms are recognized rather late [6]. Bone scans are the most sensitive routine imaging modality [2]. ^{99m}Tc labelled methylene diphosphonate (^{99m}Tc-MDP) is the most frequently used radiotracer [7]. However, with respect to the great importance of early detection in the treatment of the diseases, studies on the development of novel diagnostic methods using new radiopharmaceuticals are still investigated.

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While PET offers superior sensitivity, resolution and quantitative ability in comparison with SPECT [8], some positron emitter radiotracers mostly ^{18}F-FDG and ^{18}F-labeled NaF have been developed and utilized for bone PET-imaging [9, 10]. However, higher sensitivity for the detection of osseous metastases was reported in comparison to the other imaging modalities [11], preparation of ^{18}F needs an on-site cyclotron which restricts its application.

A generator-based ^{68}Ga radioisotope with its favorable characteristics (t ½ = 68 min, Ep [max]= 1.92 MeV) can be considered as an alternative [12]. Nowadays, ^{68}Ga has been radiolabeled with numerous molecules and applied for the detection of various abnormalities [13, 14] indicating promising results.

Bisphosphonates have a proven role in the treatment of bone metastases [15] and are the current standard therapy for reducing the frequency of skeletal-related complications [16]. Recently, radionuclides in combination with phosphonate-containing chelators, like ethylenediamine tetra (methylene phosphonic acid) (EDTMP), 1,1-hydroxyethylidene diphosphonate (HEDP) and 4-{[(bis(phosphonomethyl)) carbamoyl]methyl}-7,10 bis(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl)acetic acid (BPAMD) have been introduced to reach regions of increased bone turnover and show good results in palliative therapy of painful bone metastases as well as in diagnosis of bone metastases [17-19].

Since ^{153}Sm-EDTMP is the only FDA approved bone-seeking therapeutic radiopharmaceutical and ^{177}Lu-EDTMP has demonstrated high bone uptake and fast urinary clearance [20], optimized production of ^{68}Ga-EDTMP was reported in the recent years showing significant bone accumulation in healthy rats [21]. However, the absorbed dose of this new agent has not been reported so far.

In this study, ^{68}Ga-EDTMP was prepared and its bio-distribution in wild-type rats was studied. With respect to the importance of the absorbed dose of non-target organs in developing new radiopharmaceuticals, the human absorbed dose after injection of ^{68}Ga-EDTMP was determined for the first time based on the bio-distribution data in the rats using RAdio Detection and Ranging (RADAR) and the method of Sparks *et al.* [22].

2. Materials and Methods

2.1.Production and Quality Control of $^{68}GaCl_3$

^{68}Ge/^{68}Ga generator was eluted with 0.6 M HCL. In order to achieve $^{68}GaCl_3$ solution with high specific activity, the first fraction of the generator eluted by 0.5 mL of HCl was disregarded, and the four next fractions (2.0 mL) were considered for radiolabeling purposes.

The radionuclidic purity of the eluted ^{68}Ga from the generator was investigated by the gamma spectrometry of the decayed ^{68}Ga samples. While the content of chemical impurities was determined by Inductively Coupled Plasma (ICP-OES) method, the ITLC method was used for studying the radiochemical purity. The radiochemical purity was checked by two solvent systems of 10% ammonium acetate: methanol (1:1 V/V) and 10 mM DTPA solutions.

2.2. Preparation and Quality Control of ^{68}Ga-EDTMP

EDTMP was synthesized from phosphorous acid, ethylenediamine and formaldehyde in the presence of HCl by a modified Mannich-type reaction [14] using phosphorous acid, conc. HCl, ethylenediamine and aq. formaldehyde and recrystallization from water/methanol. Characterization of as-prepared sample was done by IR and 1HNMR methods. The results showed the bands of 3308, 2633, 2311, 1668, 1436, 1356 cm-1 at IR diagram. Also, 1HNMR (D2O, d ppm) demonstrates 3.53 (d, J = 12.3 Hz, 8H, -N-CH2-P=O), 3.85 (s, 4H, -N-CH2-). 13C NMR (D2O, d ppm):51.63, 52.73. 31P NMR (D2O, d ppm): 10.52.

^{68}Ga-EDTMP was prepared according to the previously reported procedure [21]. Briefly, 5 mg of EDTMP was added to a borosilicate vial containing a certain volume of $^{68}GaCl_3$, 352 mg of solid HEPES and 200 µl of 0.1 M acetate buffer. The vial was then heated at hot water bath with the temperature of 50-60 °C for 5 min. The radiochemical purity of the complex was then checked by ITLC method, while saline is used as a

mobile phase and Whatman No.2 paper as a stationary phase.

2.3. Biodistribution Assessment of ^{68}Ga-EDTMP in Wild-Type Rats

100 µL of final $^{68}GaCl_3$ solution was injected into the wild-type rats through their tail veins. The rats were sacrificed at specified intervals (five rats for each interval), whereas the activity and weight of their main organs were measured by an HpGe detector and a calibrated balance, respectively. %ID/g for each organ was calculated through dividing the activity of each organ at the selected time by the total injected activity and weight of the organs.

2.4.Determination of the Cumulated Activity

The non-decay corrected time activity curves for each organ were plotted, and the accumulated source activity was calculated computing the area under the curves while fitted to a mono-exponential, bi- exponential or uptake and clearance curve. In addition, the curves were extrapolated to infinity by fitting the tail of each curve to a mono-exponential curve. Then, the area under the curve was calculated.

The accumulated activity for animal organs was then extrapolated to the accumulated activity for human organs by the proposed method of Sparks *et al.* (1) [22].

$$\tilde{A}_{Human} = \tilde{A}_{Animal}$$

$$\frac{Organ\ mass\ (human) / Body\ mass\ (human)}{Organ\ mass\ (animal) / Body\ mass\ (animal)} \quad (1)$$

2.5.Calculation of Equivalent Absorbed Dose

Absorbed dose of human organs was calculated by RADAR method and according to the previously reported procedure (2) [23].

$$D = \tilde{A} \times DF \quad (2)$$

Where $\tilde{A}$ is the accumulated activity for each human organ, and DF is the parameter which represents the physical decay characteristics of the radionuclide, the range of the emitted radiations, and the organ size and configuration. In this research, DFs have been taken from the amount presented in OLINDA/EXM software.

3. Results

3.1.Production and Quality Control of $^{68}GaCl_3$

The HPGe spectrum did not show any radionuclidic impurity. The peaks of 511 and 1077 keV were only observed when originated from ^{68}Ga (Figure 1). ITLC chromatogram of $^{68}GaCl_3$ approved the radiochemical purity of higher than 99%.

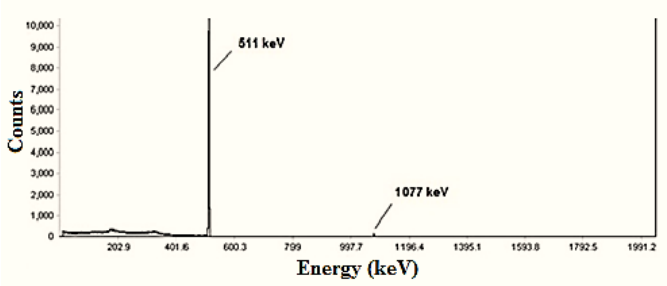

Figure 1. HPGe spectrum of $^{68}GaCl_3$

3.2.Preparation and Quality Control of ^{68}Ga-EDTMP

ITLC analysis of the final complex showed the radiochemical purity of higher than 99% (Figure 2). Using Whatman No. 2 paper and saline, the radiolabelled complex was observed at Rf of 0.9.

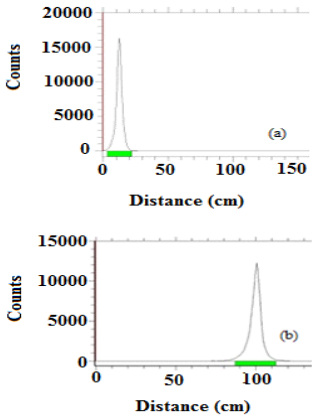

Figure 2. ITLC chromatogram of $^{68}GaCl_3$ (a) and ^{68}Ga-EDTMP (b) in saline using Whatman No. 2 as a stationary phase

3.3.Biodistribution Assessment of ^{68}Ga-EDTMP in Wild-Type Rats

The non-decay corrected %ID/g for each animal organ up to 120 m was calculated as described previously

(Figure 3). As expected, the highest amount of activity was observed in bone, while the activity in the other organs was insignificant. Approximately no activity was perceived in blood after 15 min., which shows fast clearance of activity from blood circulation. According to the results, kidney can be considered as a major excretion route.

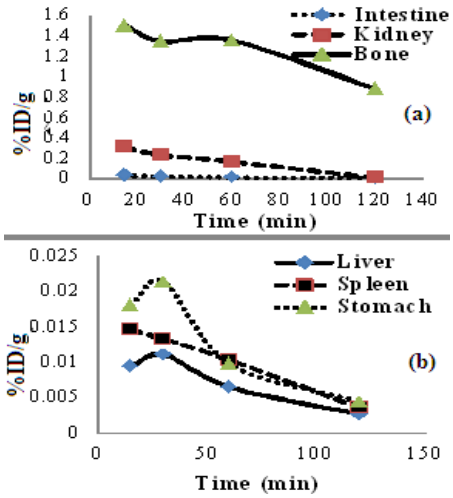

Figure 3. The non-decay corrected of the percentage of injected dose per gram (%ID/g) in intestine, kidney, bone (a), liver, Spleen and stomach (b) at 4, 24, 72 and 168 h after intravenously injection of ^{68}Ga-EDTMP into Wild-type rats

3.4.Calculation of Equivalent Absorbed Dose

The absorbed dose of human organs was computed based on the biodistribution data in Wild-type rats (Table 1). The results demonstrated an estimated absorbed dose of 0.006 mSv/MBq to the whole body. The highest equivalent absorbed dose was observed in bone surface and bone marrow with 0.112 and 0.053 mSv/MBq, respectively.

Table 1. Equivalent absorbed dose delivered into human organs after injection of ^{68}Ga-EDTMP

Target Organs	Equivalent absorbed dose in humans (mSv/MBq)
GB Wall	0.001
LLI Wall	0.003
Small Int	0.001
Stomach Wall	0.001
ULI Wall	0.001
Heart Wall	0.001
Kidneys	0.003
Liver	0.001
Lungs	0.001
Muscle	0.001
Red Marrow	0.053
Bone Surf	0.112
Spleen	0.001
UB Wall	0.001
Total Body	0.006

GW: Gallbladder Wall; LLI: lower large intestine; Int: Intestine; ULI: upper large intestine; UB Wall: Urinary Bladder Wall.

[a] Tissue weighting factors according to international commission on radiological protection, ICRP 103 (2007).

4. Discussion and Conclusion

Although higher spatial resolution and better sensitivity of PET images using ^{18}F-labeled NaF has been observed compared to the SPECT images utilizing ^{99m}Tc-MDP in many clinical trials [25, 26], absorbed dose of human organs is an important issue that needs to be addressed.

While ^{18}F decays by positron emission (97%), which yields to two 511 keV annihilation photons and electron capture (3%) with a half-life of 109.7 min., ^{99m}Tc emits photons of 140 keV energy (half-life of 6.005 h). 511 keV photons can result in the higher absorbed dose in non-target organs.

Effective dose of an adult after injection of ^{99m}Tc-MDP and ^{18}F-labeled NaF presented by ICRP report is equal to 0.0057 and 0.027 mSv/MBq, respectively [25, 26]. Although this amount for ^{18}F-labeled NaF is much higher, considering the administered activity, the effective dose will be in the same order (3.0 and 4.0 mSv for ^{99m}Tc-MDP and ^{18}F-labeled NaF, respectively).

Recently, ^{68}Ga has been introduced as a suitable radionuclide with superior characteristics, including lower half-life and its availability as a generator system in comparison with ^{18}F [24] and ^{68}Ga-EDTMP is developed for bone PET-imaging.

Based on the biodistribution study of ^{68}Ga-EDTMP in rat organs, rapid removal of the injected activity from the blood circulation is observed and the major portion of the remaining activity is accumulated into bones. Due to the high water solubility of the complex, significant excretion of the radiolabeled compound is via the kidneys.

In this study, the absorbed dose of human organs after injection of ^{68}Ga-EDTMP was calculated based on biodistribution data in rat organs. Although

extrapolation between animal data to human may lead to some over or under estimation, previous studies have indicated the usefulness of using animal biodistribution as a model for absorbed dose estimations in humans [27] and also estimation of the organ radiation exposure dose from biodistribution data in animals is a prerequisite for the clinically application of a new radiopharmaceutical [28].

Several methods have been previously proposed for extrapolating organ uptake data in animals to equivalent uptake in humans [22]. The use of these methods is seen in numerous publications [29-31].

As expected, bone surface and bone marrow with the absorbed dose of 0.112 and 0.053 mSv/MBq, respectively, received the highest absorbed dose, while the other organs received insignificant absorbed dose.

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