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Absorbed Dose Estimation of ^{68}Ga-EDTMP in Human Organs

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***Abstract*—**Bone metastases are observed in a wide range of cancers leading to intolerable pain. While early detection can help the physicians in the decision of the type of treatment, various radiopharmaceuticals using phosphonates like ^{68}Ga-EDTMP have been developed. In this work, due to the importance of absorbed dose, human absorbed dose of this new agent was calculated for the first time based on biodistribution data in Wild-type rats. ^{68}Ga was obtained from ^{68}Ge/^{68}Ga generator with radionuclidic purity and radiochemical purity of higher than 99%. The radiolabeled complex was prepared in the optimized conditions. Radiochemical purity of the radiolabeled complex was checked by instant thin layer chromatography (ITLC) method using Whatman No. 2 paper and saline. The results indicated the radiochemical purity of higher than 99%. The radiolabelled complex was injected into the Wild-type rats and its biodistribution was studied up to 120 min. As expected, major accumulation was observed in the bone. Absorbed dose of each human organ was calculated based on biodistribution in the rats using RADAR method. Bone surface and bone marrow with 0.112 and 0.053 mSv/MBq, respectively, received the highest absorbed dose. According to these results, the radiolabeled complex is a suitable and safe option for PET bone imaging.

***Keywords*—**Absorbed dose, EDTMP, ^{68}Ga, rats.

I. Introduction

BONE metastasis results from primary tumor invasion to bone. Skeletal metastasis is a common cause of severe morbidity [1] which accounts for 70% of all malignant bone tumours [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 tumour 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.

While PET offers superior sensitivity, resolution and quantitative ability in comparison with SPECT [8], some positron emitter radiotracers mostly ^{18}F-FDG 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, E_p [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 an established 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 until now.

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

II. Materials and Methods

A. 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

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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.

B. 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 ^{1}HNMR methods. The results showed the bands of 3308, 2633, 2311, 1668, 1436, 1356 cm^{-1} at IR diagram. Also, ^{1}HNMR (D_2O, d ppm) demonstrates 3.53 (d, J = 12.3 Hz, 8H, -N-CH_2-P=O), 3.85 (s, 4H, -N-CH_2-). 13C NMR (D_2O, 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.

C. 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 weigh of their main organs were measured by an HpGe detector and a calibrated balance, respectively. %ID/g for each organ was calculated by dividing the activity of each organ at the selected time to the total injected activity and weigh of the organs.

D. Determination of the Cumulated Activity

The non-decay corrected time activity curves for each organ was 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. [22] (1):

$$\tilde{A}_{\text{human organ}} = \tilde{A}_{\text{animal organ}} \frac{Organ\ mass\ (human) / Body\ mass\ (human)}{Organ\ mass\ (animal) / Body\ mass\ (animal)} \quad (1)$$

E. Calculation of Equivalent and Effective 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.

III. Results and Discussions

A. 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 that originates from ^{68}Ga (Fig. 1). ITLC chromatogram of $^{68}GaCl_3$ approved the radiochemical purity of higher than 99% (Fig. 2).

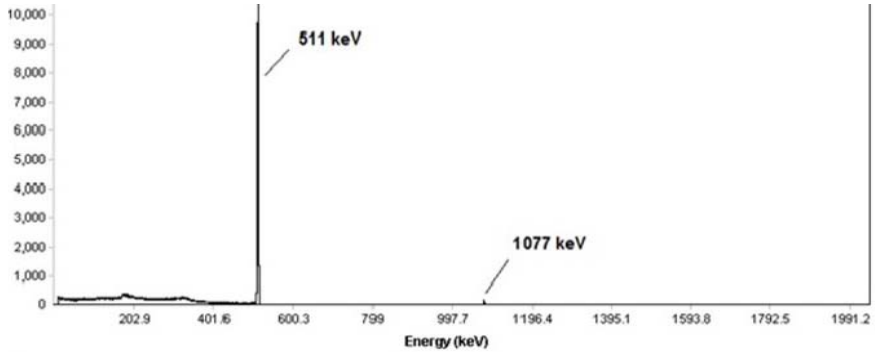

Fig. 1 HPGe spectrum of $^{68}GaCl_3$

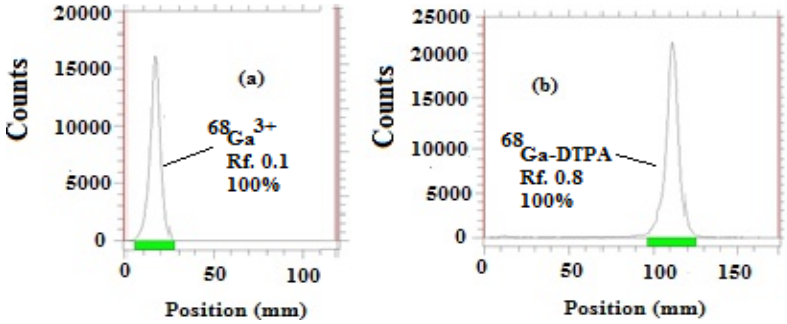

Fig. 2 ITLC chromatogram of $^{68}GaCl_3$ in 10% ammonium acetate:methanol (1:1 V/V) on silicagel sheets (a) and in 10 mM DTPA solution (pH ~4) on Whatman No. 2 paper (b)

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

ITLC analysis of the final complex showed the radiochemical purity of higher than 99% (Fig. 3). Using Whatman No. 2 paper and saline, the radiolabelled complex was observed at R_f of 0.9.

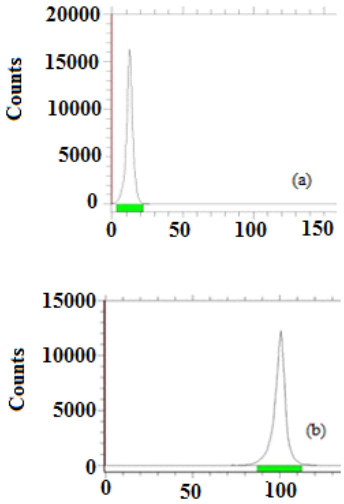

Fig. 3 ITLC chromatogram of $^{68}GaCl_3$ (a) and ^{68}Ga-EDTMP (b) in saline using Whatman No. 2 as a stationary phase

C. 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 (Fig. 4). 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 vary fast clearance of activity from blood circulation. According to the results, kidney can be considered as a major excretion route.

D. Calculation of Equivalent and Effective Absorbed Dose

The absorbed dose of human organs was computed based on the biodistribution data in Wild-type rats (Table I). 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.

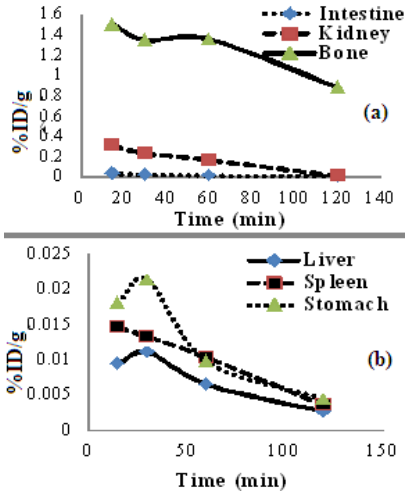

Fig. 4 The non-decay corrected of the percentage of injected dose per gram (%ID/g) in intestine, kidney, bone (a), liver, Spleen and stomach (b) at 4, 24, 72 and 168 h after intravenously injection of ^{68}Ga-EDTMP into Wild-type rats

TABLE I
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).

IV. Conclusion

In this study, ^{68}Ga-EDTMP was prepared successfully with radiochemical purity of higher than 99%. After injection of final radiolabeled complex into the Wild-type rats, considerable uptake was observed in the bone. Organs with the highest absorbed dose were bone surface and bone marrow (0.112 and 0.053 mSv/MBq, respectively), while, the other organs would not receive significant absorbed dose. Generally, the estimated absorbed dose values are much lower in comparison to the similar radiolabeled complexes.

References

[1] M. G. Agarwal and P. Nayak, "Management of skeletal metastases: An orthopaedic surgeon's guide" Indian J Orthop, vol. 49, pp. 83-100, 2015.
[2] https://radiopaedia.org/articles/skeletal-metastasis-1. Accessed on: 19/09/2017
[3] D. W. Kufe, R. E. Pollock, R. R. Weichselbaum, R. C. Bast, T. S. Gansler, J. F. Holland, et al. Holland-Frei Cancer Medicine. 6th ed. Hamilton (ON): BC Decker, 2003.
[4] H. Yousefnia, S. Zolghadri, H. R. Sadeghi, M. Naderi, A. R. Jalilian and S. Shanesazzadeh, "Preparation and biological assessment of ^{177}Lu-BPAMD as a high potential agent for bone pain palliation therapy: comparison with ^{177}Lu-EDTMP" J. Radioanl Nucl Chem, vol. 307, pp. 1243-1251, 2016.
[5] Lipton, "Pathophysiology of Bone Metastases: How This Knowledge May Lead to Therapeutic Intervention" J Support Oncol, vol. 2, pp. 205-220, 2004.
[6] H. Yousefnia, S. Zolghadri and A. R. Jalilian, "Preparation and biodistribution assessm ent of 111In-BPAMD as a novel agent for bone SPECT imagin" J Radiochim Acta, vol. 103, pp. 653-661, 2015.103(9); p. 653-661Radiochimica Acta; ISSN 0033-8230; CODEN RAACAP; v. 103(9); p. 653-661Radiochimica Acta; ISSN 0033-8230; CODEN RAACAP; v. 103(9); p. 653-661Radiochimica Acta; ISSN 0033-8230; CODEN RAACAP; v. 103(9); p. 653-661 Radiochimica Acta; ISSN 0033-8230; CODEN RAACAP; v. 103(9); p. 653-661 Radiochimica Acta; ISSN 0033-8230; CODEN RAACAP; v. 103(9); p. 653-661.
[7] S. Nilegaonkar, S.Sonar, A. Ranade, and M. Khadilkar, "TC^{99m} MDP bone scan in evaluation of painful scoliosis" Indian J Nucl Med, vol. 25, pp. 67–69, 2010.
[8] D. Cheng, Y. Wang, X. Liu, P. H.Pretorius, M. Liang, M. Rusckowski, and D. J. Hnatowich, "A comparison of ^{18}F PET and ^{99m}Tc SPECT imaging in phantoms and in tumored mice" Bioconjug Chem, vol. 21, pp. 1565–1570, 2010.
[9] B. Koolen , E. Vegt , E. J. Rutgers , W. V. Vogel, M. P. Stokkel , C. A. Hoefnagel , A. Fioole-Bruining , M. J. Vrancken Peeters and R. A. Valdés Olmos , "FDG-avid sclerotic bone metastases in breast cancer patients: a PET/CT case series" Ann Nucl Med, vol. 26, pp. 86-91, 2012.
[10] T. Uematsu , S. Yuen , S. Yukisawa , T. Aramaki , N. Morimoto , M. Endo, H. Furukawa , Y. Uchida and J. Watanabe, " Comparison of FDG PET and SPECT for detection of bone metastases in breast cancer" AJR Am J Roentgenol, vol. 184, pp1266-1273, 2005.M. Costelloe, H.H. Chuang and J. E. Madewell, "FDG PET for the detection of bone metastases: sensivity, specificity and comparison with other imaging modalities: PET Clin, vol. 5, pp. 281-295, 2010.
[12] Colleen M. Costelloe Search for articles by this author Affiliations Division of Diagnostic Imaging, Department of Diagnostic Radiology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA Correspondence Search for articles by this author Affiliations Division of Diagnostic Imaging, Department of Diagnostic Radiology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.
[13] Shetty, Y. S. Lee, and J. M. Jeong, "^{68}Ga-Labeled Radiopharmaceuticals for Positron Emission Tomography" Nucl Med Mol Imaging, vol.44, pp. 233–240, 2010.
[14] R. C. Walker, G. T. Smith, E. Liu, B. Moore, J. Clanton and M. Stabin, "Measured Human Dosimetry of ^{68}Ga-DOTATATE" J Nucl Med, vol. 54, pp. 855–860, 2013.
[15] I. Velikyan, "Prospective of ^{68}Ga-Radiopharmaceutical Development" Theranostics, vol. 4, pp. 47-80, 2014.
[16] B. Ramaswamy and C. L. shapiro,"Bisphosphonates in the Prevention and Treatment of Bone Metastases" Available at: http://www.cancernetwork.com/bone-metastases/bisphosphonates-prevention-and-treatment-bone-metastases.Accesses on: 01/09/2003
[17] Holen and R. E. Coleman, " Bisphosphonates as treatment of bone metastases" Curr Pharm Des, vol. 16, pp. 1262-1271, 2010.
[18] N. Pfannkuchen, M. Meckel, R. Bergmann, M. Bachmann,C. Bal, M. Sathekge, W. Mohnike, R. P. Baum and F. Rösch, "Novel Radiolabeled Bisphosphonates for PET Diagnosis and Endoradiotherapy of Bone Metastases" Pharmaceuticals, vol. 10, 2017.
[19] M. Fellner , R. P. Baum, V. Kubícek, P. Hermann , I. Lukes ,V. Prasad and F. Rösch , "PET/CT imaging of osteoblastic bone metastases with (68)Ga-bisphosphonates: first human study" Eur J Nucl Med Mol Imaging, vol. 37, pp. 834, 2010.
[20] Rabie, R. Enayati, H. Yousefnia, A. R, Jalilian, M. Shamsaei, S. Zolghadri, A. Bahrami-samani and M. Hosntalab, "Preparation, quality control and biodistribution assessment of ^{153}Sm-BPAMD as a novel agent for bone pain palliation therapy"Ann Nucl Med, vol. 29, pp. 870-876, 2015.
[21] H. Iagaru, E. Mittra, P. M. Colletti and H. Jadvar, "Bone-Targeted Imaging and Radionuclide Therapy in Prostate Cancer" J Nucl Med, vol. 57, pp. 19-24, 2016.
[22] Mirzaei , A. R. Jalilian, A. Badbarin, M. Mazidi, F. Mirshojaei, P. Geramifar and D. Beiki, " Optimized production and quality control of (68)Ga-EDTMP for small clinical trials" Ann Nucl Med, vol. 29, pp. 506-511, 2015.
[23] Sparks RB, Aydogan B. Comparison of the effectiveness of some common animal data scaling techniques in estimating human radiation dose. Sixth International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, TN: Oak Ridge Associated Universities; pp. 705–716, 1996.
[24] M. G. Stabin and J. A. Siegel. "Physical Models and Dose Factors for Use in Internal Dose Assessment" Health Phys, vol. 85, pp. 294-310, 2003.