

Biodistribution of ^{68}Ga -DTPMP as a potential bone-seeking imaging agent in normal rats and rats with experimental model of bone callus

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Abstract. Bone metastases are common in advanced stages of various cancers. Positron emission tomography (PET) with radiotracers based on phosphonates and gallium-68 is a promising method of bone metastases detection. In this work a new complex based on diethylene triamine pentamethylene phosphonate (DTPMP) labeled with ^{68}Ga was developed and its biodistribution in normal Wistar rats and rats with experimental model of bone callus was studied. The biodistribution studies in normal rats revealed high skeletal uptake of ^{68}Ga -DTPMP (up to 1.45 %ID/g in femur) with rapid blood clearance and minimal uptake in any other major organs, except kidneys. In rats with experimental model of bone callus the accumulation of activity in bones and soft organs and tissues was slightly lower as compared with normal rats. The amount of ^{68}Ga -DTPMP in femur with bone callus was higher than in non-lesion bones and reached 1.90 %ID/g at 2 h p.i. Therefore, ^{68}Ga -DTPMP could be a promising radiotracer for bone tumors imaging and could be a valuable alternative to ^{18}F -FDG and ^{18}F -NaF for PET centers without an onsite cyclotron.

1. Introduction

Many cancer diseases such as prostate and breast cancers may metastasize to bone in an advanced state. The major symptoms of metastatic bone lesions are severe bone pain, pathological fractures, spinal cord compression, hypercalcemia, which significantly affect the quality of life [1]. Therefore, early and accurate diagnosis of bone metastases provides the decision on the following therapy.

Skeletal metastases can be visualized via both single-photon emission computed tomography (SPECT) and positron emission tomography (PET). $^{99\text{m}}\text{Tc}$ -phosphonates have been widely used as tracers in nuclear medicine for bone scintigraphy for over thirty years [2]. However, PET has a higher diagnostic sensitivity and specificity for bone metastases detection [3]. Unfortunately, the main limitations of the most utilized PET radiotracers, fluorine-18 fluorodeoxyglucose (^{18}F -FDG) and ^{18}F -fluoride (^{18}F -NaF), are the high cost of production and the requirement of a nearby cyclotron to produce them. Moreover, ^{18}F -FDG and ^{18}F -NaF have low diagnostic impact regarding sclerotic metastases and false-positive findings in minimal degenerative changes [4].

^{68}Ga is one of the greatest practical and interesting radionuclide for clinical PET due its radiophysical properties ($T_{1/2} = 68$ min, $\beta^+ 89\%$, $E_{\beta^+ \text{ max}} = 1.9$ MeV). ^{68}Ga is a generator-produced nuclide and can be obtained from commercial $^{68}\text{Ge}/^{68}\text{Ga}$ generator at any time on demand [5]. So it



does not require an on-site cyclotron. In principle, the long half-life of the parent nuclide ^{68}Ge ($T_{1/2} = 270.8$ days) provides a long life-span generator.

Phosphonic acids and phosphonates are ideal carriers of radionuclides to bone tissue. They are known to have high affinity of phosphonate groups for calcium of hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. They are also resistant to breakdown by enzymatic hydrolysis. Combining the advantages of phosphonates and ^{68}Ga can lead to development of new bone seekers for PET and improve the detection of bone metastases.

Diethylene triamine pentamethylene phosphonate (DTPMP) is a promising ligand which can form stable complexes with many radiometals. The affinity of phosphonate ligands to bone tissue is dependent of the number of aminomethelenephosphonate groups, so it is expected that DTPMP will have the highest bone affinity.

The objective of this study was to evaluate the biodistribution of a new compound based on diethylene triamine pentamethylene phosphonate labeled with ^{68}Ga (^{68}Ga -DTPMP) as a potential bone imaging agent for PET applications in normal rats and rats with experimental model of bone callus.

2. Methods and materials

Biodistribution experiments of ^{68}Ga -DTPMP were carried out in female Wistar rats ($n = 4$ for each time point) weighing 140–160 g. All animals were divided into 2 groups. The first group of rats was injected intravenously into the tail vein of each animal with 0.37 MBq of labeled ^{68}Ga -DTPMP in a volume of 0.1 ml. The second group consisted of animals with experimental model of bone callus. The bone callus was created by fracture of right femur under anesthesia. In two weeks after fracture, when the bone callus was formed, all rats of second group received intravenously 0.37 MBq of ^{68}Ga -DTPMP in a volume of 0.1 ml. The animals of both groups were sacrificed by decapitation at 5 min, 1, 2 and 3 h after injection. The desired organs were excised, washed, placed in plastic tubes and weighted. The radioactivity in each organ was counted using gamma counter. The data are expressed as a percentage of the injected dose per gram of tissue (%ID/g).

The results from the biodistribution data for each group of mice were expressed as mean value and standard error of the mean ($M \pm m$).

3. Results and discussion

The results of ^{68}Ga -DTPMP biodistribution in normal rats and rats with experimental model of bone callus are shown in figure 1 and 2, respectively. Both groups of animals had high uptake of ^{68}Ga -DTPMP in bone tissue within 3 h post injection (p.i.). In normal rats the peak amount of activity was 1.45 %ID/g in femur, 1.17 %ID/g in tibia, 1.06 %ID/g in skull, 1.23 %ID/g in ribs, and 1.21 %ID/g in spine. The highest uptake of ^{68}Ga -DTPMP was observed in knee joint: 2.11–2.51 %ID/g. The levels of activity in skeleton of rats with experimental model of bone callus were slightly lower as compared with normal rats. Thus, femur uptake reached 1.34 %ID/g, and maximal amounts of ^{68}Ga -DTPMP in other bones were 0.99, 0.79, 1.22, 1.14, 2.49 %ID/g in tibia, skull, ribs, spine, and knee joint, respectively. The accumulation of radioactivity in femur with bone callus varied from 1.49 %ID/g at 5 min p.i. to 1.90 %ID/g at 2 h p.i. It was 1.3–1.8 times higher than in normal femur. These data indicated that ^{68}Ga -DTPMP preferentially accumulated in metabolically active bone regions.

The ratios of activity in bones and soft organs and tissue are important features of diagnostic tracers. As shown in table 1, in normal rats femur/blood and femur/muscle ratios were higher than 1 at all time points. The maximal femur/blood ratio was 3.36 at 3 h p.i., and femur/muscle ratio reached 11.72 at 2 h p.i. In rats with bone callus the amounts of activity in bone lesions were higher as compared with blood, muscle and intact femur (table 2). It is suspected the potential of ^{68}Ga -DTPMP to visualize bone metastases in vivo.

In blood of normal rats the initial concentration of ^{68}Ga -DTPMP was 1.11 %ID/g, but then it decreased rapidly to 0.43 %ID/g at 3 h p.i. In group of rats with experimental model of bone callus specific activity decreased from 0.82 %ID/g at 5 min p.i. to 0.54 %ID/g at 3 h p.i. It is known that free

$^{68}\text{Ga}^{3+}$ bounds to plasma proteins such as transferrin, ferritin, or lactoferrin [6]. Low amount of activity in blood demonstrated high stability of ^{68}Ga -DTPMP complex in vivo.

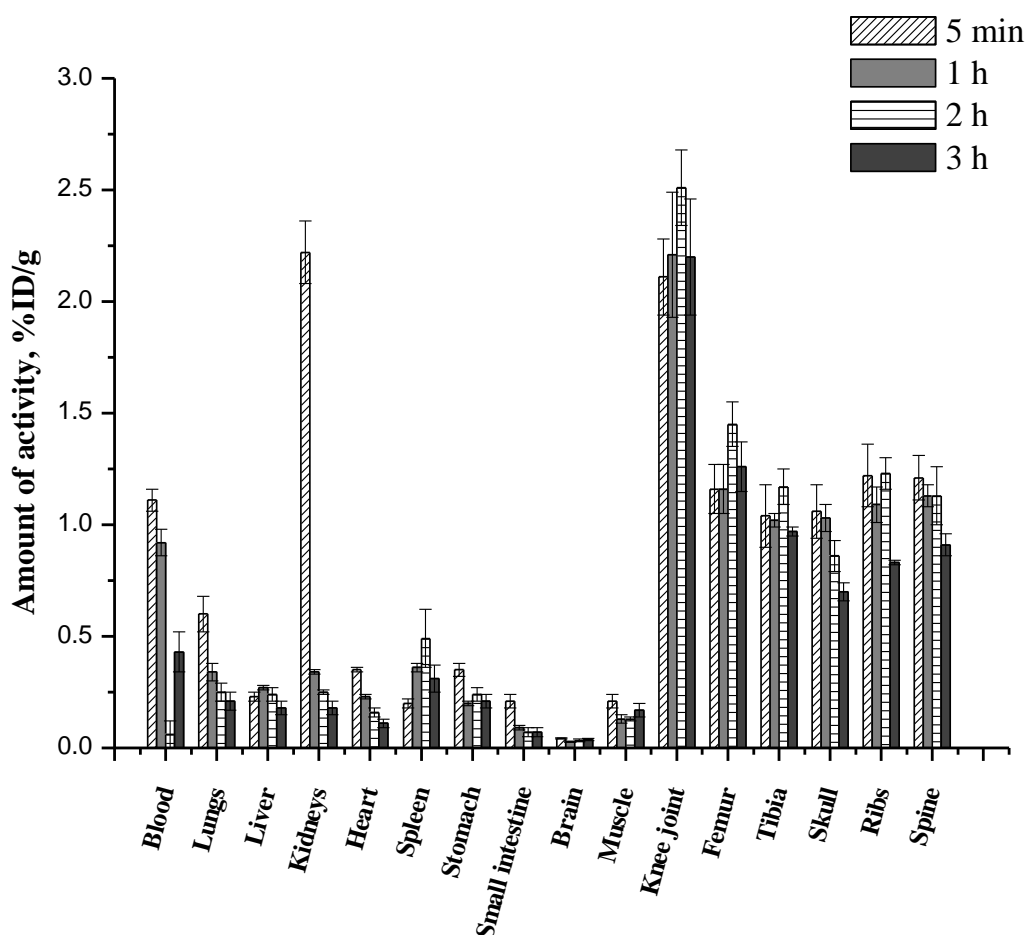


Figure 1. Biodistribution of ^{68}Ga -DTPMP in normal Wistar rats at different time points after intravenous injection

Table 1. Femur/blood and femur/muscle ratios in normal Wistar rats after intravenous injection of ^{68}Ga -DTPMP

Ratios	Time after injection			
	5 min	1 h	2 h	3 h
Femur/blood	1.05±0.08	1.27±0.10	2.53±0.36	3.36±0.72
Femur/muscle	5.74±0.45	9.10±0.61	11.72±1.42	8.46±1.62

Relatively high kidneys uptake of ^{68}Ga -DTPMP was observed in both groups of rats only at 5 min p.i., but then the level of activity in kidneys significantly decreased. In normal rats the initial amount of activity was 2.22 %ID/g, but then declined rapidly to 0.18-0.34 %ID/g. In rats with experimental model of bone callus the peak concentration of ^{68}Ga -DTPMP was 1.29 %ID/g, decreasing to 0.26-0.33 %ID/g at the next following terms. It is referred to excretion of phosphonates by the urinary system [7, 8].

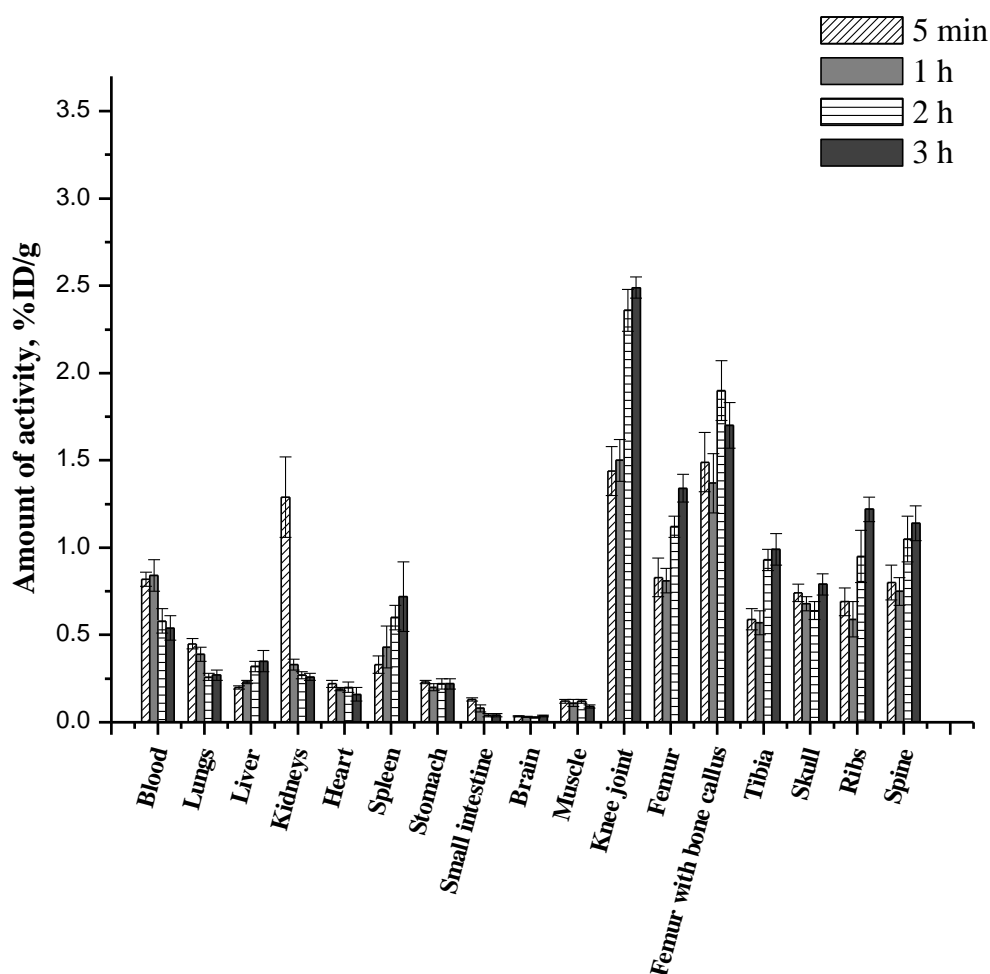


Figure 2. Biodistribution of ^{68}Ga -DTPMP in Wistar rats with experimental model of bone callus at different time points after intravenous injection

Table 2. Bone lesion/blood, bone lesion/muscle, and bone lesion/femur ratios in Wistar rats with experimental model of bone callus after intravenous injection of ^{68}Ga -DTPMP

Ratios	Time after injection			
	5 min	1 h	2 h	3 h
Bone lesion/blood	1.82±0.16	1.66±0.23	3.40±0.46	3.24±0.24
Bone lesion /muscle	12.11±1.12	13.73±2.15	16.61±1.37	19.25±2.54
Bone lesion/femur	1.81±0.10	1.67±0.10	1.70±0.17	1.28±0.12

The amounts of ^{68}Ga -DTPMP in other organs such as lungs, spleen, heart, stomach, small intestine, brain and muscle were quite low (less than 1.0 %ID/g) throughout the study. Thus, in muscle the uptake of activity was 0.18-0.27 %ID/g and 0.20-0.35 %ID/g in normal rats and rats with experimental model of bone callus, respectively. The lowest level of activity was observed in brain: less than 0.043 %ID/g.

4. Summary

The biodistribution studies in Wistar rats revealed high skeletal uptake of ^{68}Ga -DTPMP (up to 1.45 %ID/g in femur) with rapid blood clearance and minimal uptake in any other major organs, except kidneys. In rats with experimental model of bone callus the accumulation of activity in bones and soft organs and tissues was slightly lower as compared with normal rats. The amount of ^{68}Ga -DTPMP in femur with bone callus was higher than in non-lesion bones and reached 1.90 %ID/g at 2 h p.i. Therefore, ^{68}Ga -DTPMP could be a promising radiotracer for bone tumors imaging and could be a valuable alternative to ^{18}F -FDG and ^{18}F -NaF for PET centers without an onsite cyclotron.

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References

- [1] Lipton A Pathophysiology of bone metastases: how this knowledge may lead to therapeutic intervention 2004 *J. Support Oncol.* **2** 205–20
- [2] Love C, Din A S, Tomas M B, Kalapparambath T P and Palestro C J Radionuclide bone imaging: an illustrative review 2003 *Radiographics* **23**(2) 341–58
- [3] Hamaoka T, Madewell J E, Podoloff D A, Hortobagyi G N and Ueno N T Bone imaging in metastatic breast cancer 2004 *J. Clin. Oncol.* **22** 2942–53
- [4] Langsteger W, Heinisch M and Fogelman I Radiopharmaceuticals in bone, prostate and breast imaging 2006 *Semin. Nucl. Med.* **36** 73–92
- [5] Zhernosekov K P, Filosofov D V, Baum R P, Aschoff P, Bihl H, Razbash A A, Jahn M, Jennewein M and Rösch F Processing of generator-produced ^{68}Ga for medical application. 2007 *J. Nucl. Med.* **48**(10) 1741–48
- [6] Autio A, Virtanen H, Tolvanen T, Liljenbäck H, Oikonen V, Saanijoki T, Siitonen R, Käkälä M, Schüssele A, Teräs M and Roivainen A Absorption, distribution and excretion of intravenously injected $^{68}\text{Ge}/^{68}\text{Ga}$ generator eluate in healthy rats, and estimation of human radiation dosimetry 2015 *EJNMMI Research* **5** 40
- [7] Lin W Y, Lin C P, Yeh S J, Hsieh B T, Tsai Z T, Ting G, Yen T C, Wang S J, Knaoo F F Jr and Stabin M G Rhenium-188 hydroxyethylidene diphosphonate: a new generator-produced radiotherapeutic drug of potential value for the treatment of bone metastases 1997 *Eur. J. Nucl. Med.* **24**(6) 590–5
- [8] Lange R, ter Heine R, Knapp R, de Klerk JMH, Bloemendal H J and Hendrikse N H Pharmaceutical and clinical development of phosphonate-based radiopharmaceuticals for the targeted treatment of bone metastases 2016 *Bone* **91** 159–79