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V M Petriev[1,2], V K Tishchenko[1], A A Mikhailovskaya[1], E D Stepchenkova[1], K A Kuzenkova[1], A A Postnov[2] and I N Zavestovskaya[2]

[1] Tsyb Medical Radiological Research Centre, Obninsk, Russia
[2] National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Moscow, Russia
e-mail: petriev@mrrc.obninsk.ru

Abstract. ^{68}Ga-labeled phosphonates are of great interest due to their possibility to serve promising agents in nuclear medicine for bone tissue PET imaging. It is known that multidentate aminophosphonate ligands could form much stable chelates with different radiometals as compared to diphosphonates. In this work we studied the pharmacokinetic properties of ^{68}Ga-labeled complexes with two, four and five phosphonate groups (^{68}Ga-HEDP, ^{68}Ga-EDTMP, and ^{68}Ga-DTPMP, respectively) in normal Wistar rats after intravenous administration. It was shown that the structure of phosphonates had a great influence on the biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, and ^{68}Ga-DTPMP. Complexes with higher number of aminomethylenephosphonate groups (^{68}Ga-EDTMP and ^{68}Ga-DTPMP) had higher bone uptake than diphosphonate ^{68}Ga-HEDP. In blood ^{68}Ga-HEDP had lower activity than ^{68}Ga-EDTMP and ^{68}Ga-DTPMP, indicating poor stability of diphosphonate-based complex. In other soft organs and tissues ^{68}Ga-EDTMP and ^{68}Ga-DTPMP uptake was slightly lower as compared with ^{68}Ga-HEDP. In conclusion, ^{68}Ga-EDTMP and ^{68}Ga-DTPMP have the potential to be suitable radiotracers for bone tissue PET imaging.

1. Introduction

About 65-75 % of the patients, especially with advanced breast and prostate cancer, suffer from skeletal metastases [1]. Among the consequences of bone metastases are pathologic fractures, hypercalcemia, spinal cord compression, and other nerve-compression complications associated with severe and persisting pain. Early detection and accurate assessment of bone lesions are required because bone metastases are an important factor in treatment and prognosis of cancer disease.

Bone scintigraphy is the easiest and cheapest method to detect bone metastases. For this purpose several ^{99m}Tc-labeled phosphonates are available, including ^{99m}Tc-methylenediphosphonate (^{99m}Tc-MDP), and ^{99m}Tc-hydroxymethylene diphosphonate (^{99m}Tc-HMDP). Positron emission tomography with fluorine-18 fluorodeoxyglucose (^{18}F-FDG) and ^{18}F-fluoride, is also an important tool for detecting bone metastases with better resolution and sensitivity [2]. Moreover, hybrid imaging technology, which combines PET with computer tomography, is certainly superior in the detection of bone lesions. In detecting osteolytic bone metastases, ^{18}F-FDG PET/CT has been reported to be superior to bone scintigraphy [3].

The source of the ^{18}F is a proton medical cyclotron with particle energies from 10 to 20 MeV range which limits the availability of the radionuclide to the PET centers [4]. In opposition to ^{18}F, gallium-68 doesn't require the use cyclotrons due to its easy availability from the commercial $^{68}Ge/^{68}Ga$ generator. The optimal radiation properties of ^{68}Ga ($T_{1/2}$ = 68 min, β+89 %, $E_{\beta\ max}$ = 1.9 MeV) and the long half-life of the parent nuclide ^{68}Ge ($T_{1/2}$ = 271 days) allows long-term PET imaging on-site. It also provides sufficient levels of radioactivity for high quality images, short scanning while minimizing the radiation dose to the patient and personnel. It also allows repetitive examinations within the same day [5].

Successful radionuclide imaging of skeletal metastases is based on selective accumulation and prolonged retention of the radiopharmaceutical at the skeletal lesions. Phosphonates are ideal carriers of radioactivity to bone tissue. Their high affinity towards bone is based on their ability to become incorporated into the hydroxyapatite crystal by chemisorption onto the surface of bone. Therefore, the development of new compounds based on phosphonates and ^{68}Ga may improve the quality of bone metastases detection.

The objective of this study was to compare the biodistribution of different phosphonate-based complexes labeled with ^{68}Ga as potential radiotracers for bone metastases visualization by PET.

2. Methods and materials

Biodistribution experiments were performed in female Wistar rats (n = 4 for each time point) weighing 140–160 g. All animals were divided into 4 equal groups. The first group of rats was injected through the tail vein of each animal with 0.37 MBq of labeled ^{68}Ga-hydroxyethylidene diphosphonate (^{68}Ga-HEDP) in a volume of 0.1 ml. The animals of second group were injected intravenously with 0.37 MBq of ^{68}Ga-N,N,N',N'-ethylenediaminetetrakis(methylene phosphonic acid) (^{68}Ga-EDTMP) in a volume of 0.1 ml. The third group of animals was injected intravenously with 0.37 MBq of ^{68}Ga-diethylenetriaminepentamethylene phosphonate (^{68}Ga-DTPMP) in a volume of 0.1 ml. The fourth group was a control: each animal received intravenous injection of 0.37 MBq of $^{68}GaCl_3$ in a volume of 0.1 ml.

All animals were sacrificed by decapitation at 5 min, 1, 2 and 3 h post-injection (p.i.). 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 ± m). Student's t test was used to analyze data throughout all studies between groups at different time points, and $p \leq 0.05$ was considered statistically significant.

3. Results and discussion

The results of ^{68}Ga-HEDP, ^{68}Ga-EDTMP and ^{68}Ga-DTPMP biodistribution in bone tissue are shown in figure 1. It was found that ^{68}Ga-EDTMP and ^{68}Ga-DTPMP had rapid and significant bone uptake, and also had much higher uptake than the soft organs and tissues studied. On the contrary, skeletal accumulation of ^{68}Ga-HEDP was as low as $^{68}GaCl_3$. The highest amounts of all complexes were in knee joints: up to 2.87 %ID/g for ^{68}Ga-EDTMP, 2.51 %ID/g for ^{68}Ga-DTPMP, and only 1.52 %ID/g for ^{68}Ga-HEDP. In femur the specific activity of ^{68}Ga-EDTMP was 1.05-1.61 %ID/g, ^{68}Ga-DTPMP – 0.81-1.34 %ID/g, and ^{68}Ga-HEDP – 0.41-0.91 %ID/g. For ^{68}Ga-EDTMP and ^{68}Ga-DTPMP high bone uptake was observed already at 5 min p.i., reached the maximal values at 1-2 h p.i., and then slightly decreased. In other bones (tibia, skull, ribs, and spine) the levels of radioactivity were lower as compared with knee joints and femur.

It is known that phosphonic groups are responsible for the binding with hydroxyapatite of bone tissue [6]. Besides, they are also involved in the complexation with metal ions [7]. Therefore, multidentate aminophosphonate ligands is considered to form much stable chelates with different radiometals as compared to diphosphonates. In our work we showed that complexes with four and five

aminomethylenephosphonate groups (^{68}Ga-EDTMP and ^{68}Ga-DTPMP) had higher bone uptake than diphosphonate-based complex (^{68}Ga-HEDP).

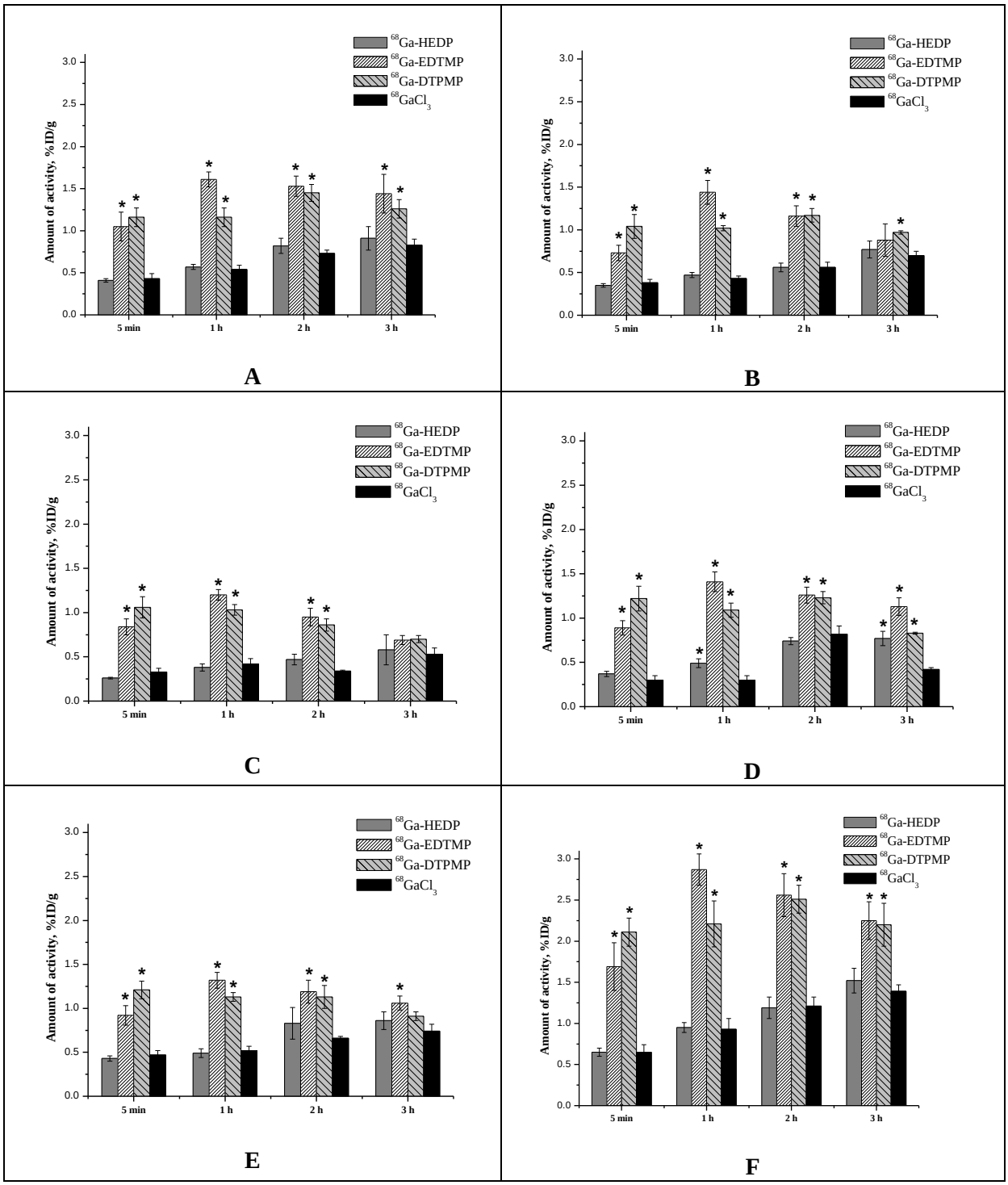

Figure 1. Biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, ^{68}Ga-DTPMP, and $^{68}GaCl_3$ in femur (A), tibia (B), skull (C), ribs (D), spine (E), and knee joint (F) of Wistar rats at different time points after intravenous injection
* – $p \leq 0,05$ as compared with control group

As shown in figure 2 A, in blood the amounts of ^{68}Ga-EDTMP and ^{68}Ga-DTPMP were lower than $^{68}GaCl_3$ ($p < 0.05$). Initial level of ^{68}Ga-EDTMP was 1.36 %ID/g, decreasing rapidly to 0.57 %ID/g. Specific activity of ^{68}Ga-DTPMP declined from 1.11 to 0.43 %ID/g within 3 hours, whereas the activity of ^{68}Ga-HEDP was as high as 0.83-1.75 %ID/g. It was reported that that free $^{68}Ga^{3+}$ bounds to plasma proteins such as transferrin, ferritin, and lactoferrin [8]. For this reason $^{68}GaCl_3$ had high and prolonged retention in blood throughout the study (1.36-2.06 %ID/g). High amount of activity in blood demonstrated low stability of ^{68}Ga-HEDP complex in vivo. The incorporation of ^{68}Ga into EDTMP and DTPMP reduced the amount of radioactivity in blood, indicating high stability of ^{68}Ga-EDTMP and ^{68}Ga-DTPMP complexes.

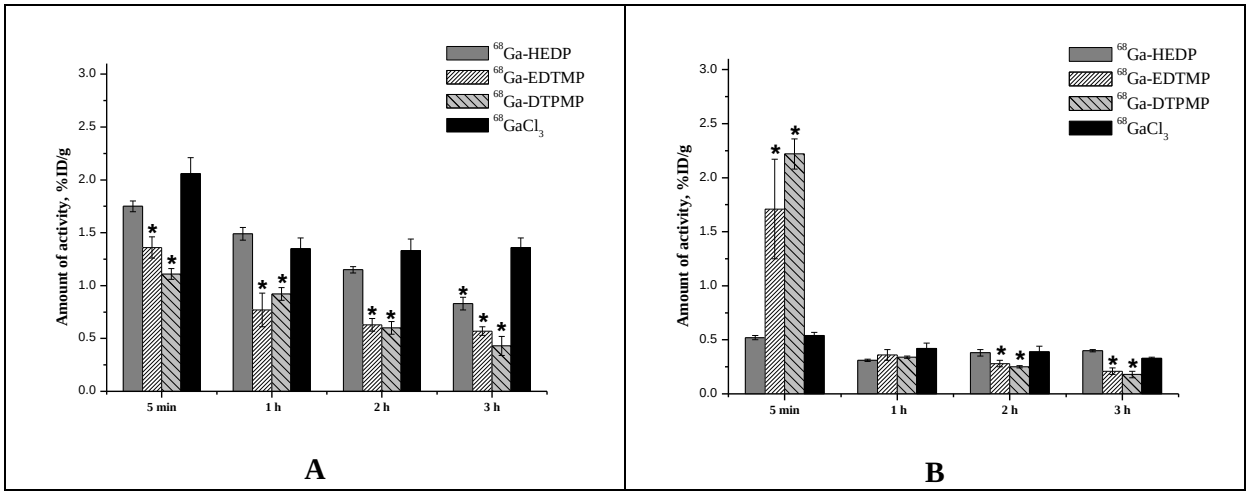

Figure 2. Biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, ^{68}Ga-DTPMP, and $^{68}GaCl_3$ in blood (A) and kidneys (B) of Wistar rats at different time points after intravenous injection
* – $p \leq 0,05$ as compared with control group

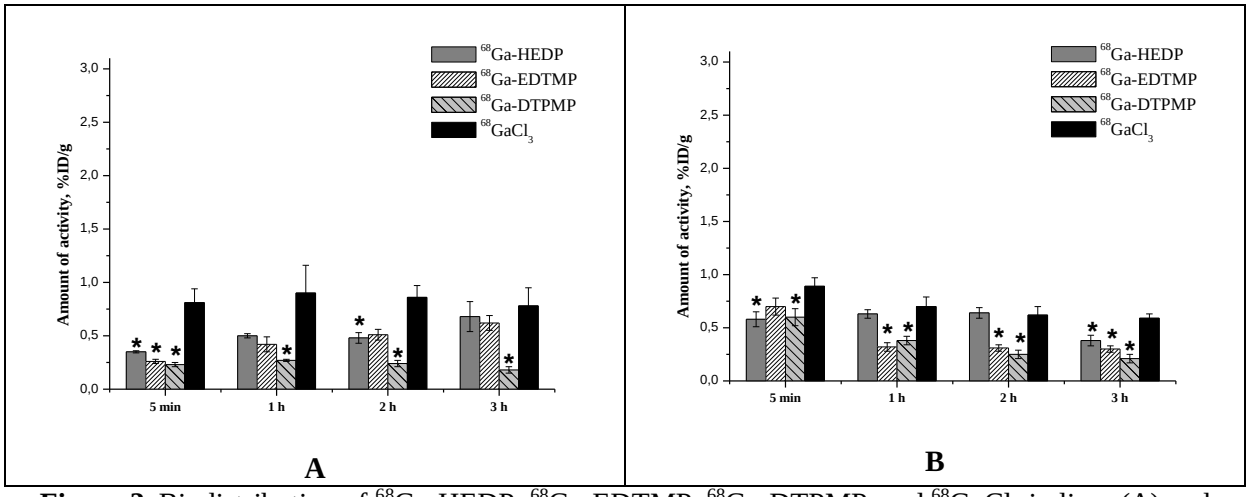

Figure 3. Biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, ^{68}Ga-DTPMP, and $^{68}GaCl_3$ in liver (A) and lungs (B) of Wistar rats at different time points after intravenous injection
* – $p \leq 0,05$ as compared with control group

A comparative biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, ^{68}Ga-DTPMP, and $^{68}GaCl_3$ are presented in figure 2B. ^{68}Ga-EDTMP and ^{68}Ga-DTPMP revealed high kidney uptake (1.71 and 2.22

%ID/g for ^{68}Ga-EDTMP and ^{68}Ga-DTPMP, respectively) at 5 min p.i. It is explained by excretion of phosphonates through the urinary system [9]. Later the concentration of both complexes decreased considerably and was less than 0.5 %ID/g. The behavior of ^{68}Ga-HEDP was similar to $^{68}GaCl_3$, and the amount of activity didn't exceed 0.52 %ID/g.

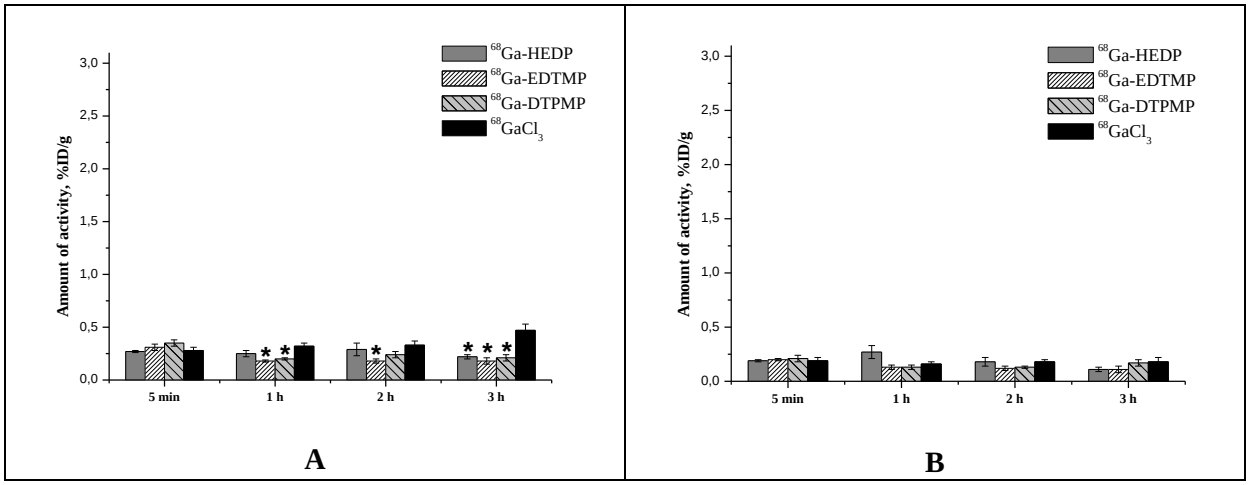

Figure 4. Biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, ^{68}Ga-DTPMP, and $^{68}GaCl_3$ in stomach (A) and muscle (B) of Wistar rats at different time points after intravenous injection
* – $p \leq 0{,}05$ as compared with control group

The amounts of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, ^{68}Ga-DTPMP in other organs such as liver, lungs, spleen, heart, stomach, small intestine, brain and muscle were quite low (less than 1 %ID/g) throughout the study. Besides, after intravenous injection of ^{68}Ga-labeled phosphonate complexes the levels of activity in these organs were lower or equal as compared with $^{68}GaCl_3$ biodistribution.

In liver the uptake of ^{68}Ga-DTPMP decreased and was lower than $^{68}GaCl_3$ throughout the study ($p < 0.05$). The activities of ^{68}Ga-HEDP and ^{68}Ga-EDTMP were lower than $^{68}GaCl_3$ only at 5 min p.i., but then increased up to 0.68 and 0.62 %ID/g for ^{68}Ga-HEDP and ^{68}Ga-EDTMP, respectively (figure 3A). In lungs the amounts of ^{68}Ga-EDTMP and ^{68}Ga-DTPMP declined rapidly, whereas ^{68}Ga-HEDP excreted slower (figure 3B).

The levels of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, and ^{68}Ga-DTPMP in stomach fell about 2 times during the study. In contrast, the uptake of $^{68}GaCl_3$ increased (figure 4A). In muscle, specific activity of ^{68}Ga-labeled phosphonate complexes didn't exceed 0.27 %ID/g for ^{68}Ga-HEDP, and 0.20 and 0.21 %ID/g for ^{68}Ga-EDTMP and ^{68}Ga-DTPMP, respectively. The uptake of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, and ^{68}Ga-DTPMP had no significant differences with $^{68}GaCl_3$ uptake (figure 4B).

4. Summary

Therefore, the structure of phosphonates has a great influence on the biodistribution of ^{68}Ga-HEDP, ^{68}Ga-EDTMP, and ^{68}Ga-DTPMP. Complexes with higher number of aminomethylenephosphonate groups had higher bone uptake than diphosphonate. Thus, amounts of ^{68}Ga-EDTMP and ^{68}Ga-DTPMP in femur reached 1.61 and 1.34 %ID/g, whereas the highest activity of ^{68}Ga-HEDP was only 0.91 %ID/g. In blood ^{68}Ga-HEDP had lower activity than ^{68}Ga-EDTMP and ^{68}Ga-DTPMP, indicating poor stability of diphosphonate-based complex. In other soft organs and tissues ^{68}Ga-EDTMP and ^{68}Ga-DTPMP uptake was slightly lower as compared with ^{68}Ga-HEDP. In conclusion, ^{68}Ga-EDTMP and ^{68}Ga-DTPMP have the potential to be suitable radiotracers for bone tissue PET imaging.

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