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The biodistribution of a new bone-seeking agent based on pentaphosphonic acid and gallium-68 in tumor-bearing rats

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Abstract. Many tumors are associated with the occurrence of bone metastases, so skeletal imaging, especially by positron emission tomography (PET), is a major problem in clinical nuclear medicine. Gallium-68 (⁶⁸Ga) is an interesting radionuclide for PET because of its appropriate radiophysical properties. Phosphonates are known to be selectively accumulated in bone tissue, so they can be ideal carriers of radionuclides. The present study was devoted to development of a new compound based on pentaphosphonic acid labeled with ⁶⁸Ga (⁶⁸Ga-PPA) as a potential bone imaging agent for PET applications and evaluation of its biodistribution in Wistar rats with subcutaneously transplanted cholangioma RS-1. Biodistribution studies of ⁶⁸Ga-PPA demonstrated rapid and selective bone accumulation and low uptake in any of the major organs and tissues. A total uptake of ⁶⁸Ga-PPA in skeleton reached 31.17±2.84 %ID and retained at the same level until the end of the study. However, the amount of ⁶⁸Ga-PPA in bone tissue was slightly lower as compared with free ⁶⁸Ga³⁺, but lower level of ⁶⁸Ga-PPA in blood made it more suitable for diagnostic purposes. In conclusion, ⁶⁸Ga-PPA may serve a promising agent in nuclear medicine for bone tissue PET imaging.

1. Introduction

Besides lung and liver, bones are common sites for the development of metastases. They are often complicated by hypercalcemia, pathological fractures, spinal cord compression, severe bone pain that can significantly decrease the quality of life and, therefore, result in shorter survival. So accurate diagnosis of bone metastases at an early stage is of great importance for patients.

Bone metastases can be visualized via both single-photon emission computed tomography (SPECT) and positron emission tomography (PET). These imaging techniques are non-invasive methods, which allow diagnosis, staging, response assessment and subsequent tumor surveillance during follow-up [1]. They are known to detect pathological changes in bones before occurrence of anatomical changes. Phosphonate complexes of ⁹⁹mTc such as MDP (methylenediphosphonate) and DPD (3,3-diphosphono-1,2-propandicarboxylic acid) are already used as SPECT tracers [2]. In contrast to SPECT, PET offers a higher spatial resolution, sensitivity, and accurate signal quantification [3]. These advantages of PET are crucial, especially in the case of small size lesions. Unfortunately, the production of the most utilized PET radiotracers, fluorine-18 fluorodeoxyglucose (¹⁸F-FDG) and ¹⁸F-fluoride, is dependent on the availability of a nearby cyclotron.
Phosphonates and phosphonic acids show high affinity for hydroxyapatite Ca_{10}(PO_4)_6(OH)_2 – a main inorganic component of bone tissue. They are stable against chemical and enzymatic hydrolysis [4]. For these reasons phosphonates can serve as ideal carriers of radionuclides to bone tissue.

Gallium-68 (^{68}\text{Ga}) is a potential radionuclide for PET imaging. ^{68}\text{Ga} possess a half-life of 68 min, positron emission fraction of 89% and  E_{\beta}^\text{max} \approx 1.9 \text{ MeV}. It also can be easily obtained on-site from commercial ^{68}\text{Ge}/^{68}\text{Ga} generators without use of a cyclotron [5]. Besides, the use of a ^{68}\text{Ge}/^{68}\text{Ga} generator system ensures direct access to ^{68}\text{Ga} for a period of up to one year [5]. ^{68}\text{Ga}^{3+} cation can form stable complexes with many ligands containing oxygen and nitrogen as donor atoms. This makes ^{68}\text{Ga} suitable for complexation with chelators and various macromolecules, allowing for kit development [6]. Development of ^{68}\text{Ga}-based phosphonate derivatives for bone PET, which can serve as an alternative to already available radiopharmaceuticals, may provide further improvement in bone imaging.

The objective of this study was to evaluate the biodistribution of a new compound based on pentaphosphonic acid labeled with ^{68}\text{Ga} (^{68}\text{Ga}-PPA) as a potential bone imaging agent for PET applications in tumor-bearing rats and compare with ^{68}\text{GaCl}_3 biodistribution.

2. Methods and materials

Biodistribution experiments of both ^{68}\text{Ga}-PPA and ^{68}\text{GaCl}_3 were carried out in female Wistar rats (n = 4 for each time point) weighing 140–160 g with subcutaneously transplanted cholangioma RS-1. To get a solid form of cholangioma RS-1 the donor rat with tumor was killed by cervical disruption, the tumor tissue was isolated, ground up, diluted in physiological saline and implanted subcutaneously into right flanks of Wistar rats (100 mg/rat in a volume of 0.1 ml). When the tumor volume reached 0.7-0.8 cm³, the rats were used for biodistribution experiments.

All animals were divided into two groups. The first group of rats was injected intravenously into the tail vein of each animal with 0.37 MBq of labeled ^{68}\text{Ga}-PPA complex in a volume of 0.1 ml. The second group received intravenously 0.37 MBq of ^{68}\text{GaCl}_3 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 collected, washed with normal saline 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). Activity in the femur was considered for obtaining the total skeletal uptake assuming the skeleton to be 10% of the total body weight.

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<0.05 was considered statistically significant.

3. Results and discussion

The results of biodistribution experiments are presented in figure 1. It was shown that ^{68}\text{Ga}-PPA had high bone affinity. Femur uptake of ^{68}\text{Ga}-PPA increased from 0.76±0.20 %ID/g at 5 min postinjection (p.i.) to 1.47±0.13 %ID/g at 1 h and then slightly decreased to 1.39±0.05 at 2 h and 1.40±0.13 %ID/g at 3 h p.i. The amount of ^{68}\text{GaCl}_3 in femur was higher as compared with ^{68}\text{Ga}-PPA, as shown in figure 2, and reached 3.03±0.62 %ID/g at 1 h p.i. It is known that ^{68}\text{GaCl}_3 binds to hydroxyapatite, human cortical matrix and demineralised bone matrix (Toegel S, Wadsak W, et al., 2008), but ^{68}\text{GaCl}_3 isn’t suitable for bone lesions imaging because of its high binding affinity to blood protein (e.g. transferrin, lactoferrin, ferritin).

Total amount of ^{68}\text{Ga}-PPA in skeleton was slightly lower than that of ^{68}\text{GaCl}_3. As shown in figure 3, the maximum uptake of ^{68}\text{Ga}-PPA was 31.17±2.84 %ID at 1 h p.i. and then retained at the level of 28.94±1.14 %ID and 28.63±1.38 %ID at 2 and 3 h p.i., respectively. The concentration of ^{68}\text{GaCl}_3 raised from 11.21±3.60 %ID at 5 min p.i. to 40.56±3.84 %ID at 3 h p.i.

High uptake of ^{68}\text{GaCl}_3 (up to 3.64±0.28 %ID/g) was observed in blood. After intravenous injection, the ^{68}\text{Ga} radioactivity can migrate in the blood circulation as free ^{68}\text{Ga}^{3+} or ^{68}\text{Ga}^{3+} bound to transferrin, ferritin, or lactoferrin [8]. The incorporation of ^{68}\text{Ga} into the PPA apparently reduced the
amount of radioactivity in blood. Thus, the amounts of $^{68}$Ga-PPA in blood were approximately 2–5 times lower as compared with $^{68}$GaCl$_3$ and didn’t exceed 0.70±0.08 %ID/g at 5 min p.i. (figure 4).

![Figure 1. Biodistribution of $^{68}$Ga-PPA in Wistar rats with cholangioma RS-1 at different time points after intravenous injection.](image1)

Tumor uptake of $^{68}$Ga-PPA was almost similar to $^{68}$GaCl$_3$. As shown in figure 5, the highest uptakes of $^{68}$Ga-PPA and $^{68}$GaCl$_3$ were 0.63±0.24 %ID/g and 0.34±0.07 %ID/g at 5 min p.i., respectively. Tumors are characterized by higher density of newly formed blood vessels compared to non-tumor tissue. Tumor vessels are more leaky due to the discontinuous endothelium and greater
vascular permeability secondary to the elevated levels of vasoactive and growth factors [9]. That’s why $^{68}$Ga-PPA and $^{68}$GaCl$_3$ non-specifically accumulate in tumor.

![Figure 4](image4.png)

**Figure 4.** Specific amounts of radioactivity in blood of Wistar rats with cholangioma RS-1 after intravenous injection of $^{68}$Ga-PPA and $^{68}$GaCl$_3$.

Biodistribution of $^{68}$Ga-PPA and $^{68}$GaCl$_3$ in liver and kidneys are presented in figures 6 and 7. Liver and kidney uptake of $^{68}$Ga-PPA was significantly lower than that of $^{68}$GaCl$_3$. Relatively high kidneys uptake of $^{68}$Ga-PPA (up to 1.03±0.31 %ID/g) was observed only at 5 min p.i., but then the level of activity in kidneys decreased 3 fold and was 0.28–0.33 %ID/g.

![Figure 5](image5.png)

**Figure 5.** Specific amounts of radioactivity in tumor of Wistar rats with cholangioma RS-1 after intravenous injection of $^{68}$Ga-PPA and $^{68}$GaCl$_3$.

![Figure 6](image6.png)

**Figure 6.** Specific amounts of radioactivity in liver of Wistar rats with cholangioma RS-1 after intravenous injection of $^{68}$Ga-PPA and $^{68}$GaCl$_3$.

![Figure 7](image7.png)

**Figure 7.** Specific amounts of radioactivity in kidneys of Wistar rats with cholangioma RS-1 after intravenous injection of $^{68}$Ga-PPA and $^{68}$GaCl$_3$.

The amounts of $^{68}$Ga-PPA in other organs such as 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-PPA the levels of activity in these organs were lower or equal as compared with $^{68}$GaCl$_3$ biodistribution.
4. Summary

Biodistribution studies of $^{68}$Ga-PPA in tumor-bearing Wistar rats demonstrated rapid and selective bone accumulation and low uptake in any of the major organs and tissues. A total uptake of $^{68}$Ga-PPA in skeleton reached 31.17±2.84 %ID and retained at the same level until the end of the study. However, the amount of $^{68}$Ga-PPA in bone tissue was slightly lower as compared with free $^{68}$Ga$^{3+}$, but lower level of $^{68}$Ga-PPA in blood made it more suitable for diagnostic purposes. In conclusion, $^{68}$Ga-PPA may serve a promising agent in nuclear medicine for bone tissue PET imaging.

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