The influence of temperature on biodistribution of N,N,N’,N’-ethylenediaminetetrakis(methylene phosphonic) acid labeled with gallium-68

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Abstract. Bone metastases are serious complication in the progression of various types of cancer. It determines the requirement of modern nuclear diagnostic tools, including positron emission tomography (PET). In this study the biodistribution of EDTMP labeled with gallium-68 ($^{68}$Ga-EDTMP) prepared at different temperature (20, 50, and 95 °C) was investigated. All experimental studies were performed in healthy intact Wistar rats by measuring the radioactivity in organs and tissues with gamma counter. All $^{68}$Ga-EDTMP formulations accumulated predominantly in bones. Only in tibia the uptake of $^{68}$Ga-EDTMP prepared at 95 °C was higher (p < 0.05) than $^{68}$Ga-EDTMP prepared at 20 °C, but in other bones there weren’t any statistical differences in uptake of $^{68}$Ga-EDTMP formulations. The amounts of $^{68}$Ga-EDTMP formulations in soft organs and tissues were lower when compared with bones. In conclusion, a temperature of reaction mixture had an influence on the biodistribution of $^{68}$Ga-EDTMP in bones.

1. Introduction

Many tumors are associated with bone metastases development. These bone metastases can cause severe complications, such as pathological bone fractures and bone pain. It is important to detect bone metastases as early as possible for treatment planning. One of the most important medical imaging techniques for assessing bone metastases throughout the whole body is radionuclide bone imaging with bone-seeking radiopharmaceuticals [1].

The major part of radiopharmaceuticals contains phosphonates or its derivatives, which have high affinity to hydroxyapatite of bones. They are known to accumulate mainly in bones, especially in inflammatory, destructive and metastatic lesions. Therefore, phosphonates are ideal carriers of radionuclides to bone tissue.

There are three commercially available radiopharmaceuticals for bone imaging in Russia. All of them are labeled with technetium-99m, so lesions can be detected by conventional imaging or single photon emission computed tomography (SPECT). Positron emission tomography (PET) is considered to have higher sensitivity and spatial resolution that allow improving quality of bone metastases imaging. Unfortunately, the application of $^{18}$F-sodium fluoride ($^{18}$F-NaF) and non-specific bone tracer 2-deoxy-2-[$^{18}$F]fluoro-glucose ($^{18}$F-FDG) is determined by local cyclotron availability.

The development of radiopharmaceuticals based on generator produced radionuclides can help to overcome the drawbacks of $^{18}$F-containing agents. Gallium-68 is a positron-emitting radionuclide ($T_{1/2}$...
= 67.7 min, β+ = 89%, E_{max} = 1.9 MeV) with significant clinical imaging potential (Suzuki K, Satake M, et al., 2011). It can be produced on-site from the $^{68}$Ge/$^{68}$Ga generator for 12-18 months. Modern $^{68}$Ge/$^{68}$Ga generator was introduced in early 2000, and then $^{68}$Ga-radiotherapeutics became to attract great interest [2]. So far most of the $^{68}$Ga-labeled bisphosphonates studied were indirectly chelated to the binding moiety [2, 3]. N,N,N’,N’-ethylenediaminetetraakis(methylene phosphonic) acid (EDTMP) is a suitable ligand for the development of diagnostic or therapeutic bone-seeking radiopharmaceuticals. $^{68}$Ga is complexed with EDTMP without chelator. Nowadays, due to the rising number of PET scanners, $^{68}$Ga-EDTMP represents a convenient agent for PET bone scanning [4]. $^{68}$Ga-EDTMP has high bone uptake in healthy bones and in bone callus of Wistar rats [5]. We have already shown that temperature had an impact on skeletal uptake of the monopotassium salt of hydroxymethylidenediphosphate labeled with $^{188}$Re [6]. In this study we synthesized three $^{68}$Ga-N,N,N’,N’-ethylenediaminetetraakis(methylene phosphonic) acid ($^{68}$Ga-EDTMP) formulations at different temperature (20 °C, 50 °C and 95 °C) and compared their biodistribution in vivo.

2. Materials and methods

$^{68}$Ga/$^{68}$Ge generator was obtained from Cyclotron Co., Ltd (Obninsk, Russia). Preparation of $^{68}$Ga-EDTMP was the following: 10 mg of EDTMP was placed in the vial of 10 ml volume and dissolved in 1.5 ml of bidistilled water. Then 0.5 ml of $^{68}$GaCl$_3$ in 0.05 M of HCl solution (37 MBq) added and adjusted with 0.1 M of sodium bicarbonate solution to pH of 4.0. The volume of mixture was brought up to 4.0 ml with bidistilled water. Three vials with $^{68}$Ga-EDTMP were obtained. Two of them were heating at 50 °C or 95 °C with stirring for 10 minutes and then were cooled to room temperature. Then mixture was filtered through a 0.22 μm membrane filter.

Radiochemical impurities were detected by paper chromatography and did not exceed 5%.

All animal studies were carried out in intact healthy female Wistar rats (140-160 g). Animals were divided into 3 equal groups (n = 16 for each group). Every rat was intravenously administrated with 0.18-0.37 MBq of $^{68}$Ga-EDTMP formulations prepared at 20 °C, 50 °C or 95 °C respectively in a volume of 0.1 ml. Animals were sacrificed at 5 min, 1, 2 and 3 h after injection (n = 4 for each time interval). The organs of interest were isolated and weighed. The radioactivity was measured by automatic gamma counter. The uptake was calculated as a percentage of the injected dose per gram of tissue (%ID/g). All animal experiments were carried out in strict compliance with the national laws related to the conduct of animal experiments.

The results of the biodistribution data for each group of rats were expressed as mean value and standard error of the mean (M ± m). Comparisons between groups at different time points were analyzed using the Kruskal-Wallis test, followed by Dunn’s test, with a significance level of 5%.

3. Results and discussion

The uptake of $^{68}$Ga-EDTMP formulations prepared at different temperature is represented in figure 1. Biodistribution experiments showed that all $^{68}$Ga-EDTMP formulations accumulated mainly in bones and knee joints. The amount of $^{68}$Ga-EDTMP prepared at 20 °C in the knee joint was 1.69-2.87 % ID/g, when the uptake of another formulations was 1.23-2.04 % ID/g and 1.60-2.38 % ID/g for $^{68}$Ga-EDTMP prepared at 50 and 95 °C, respectively.

In tibia the uptake of $^{68}$Ga-EDTMP prepared at 95 °C was higher when compared with $^{68}$Ga-EDTMP prepared at 20 °C (p < 0.05) and reached 2.02±0.08 % ID/g at 2 h postinjection (p.i.). The maximal amounts of $^{68}$Ga-EDTMP formulations prepared at 20 °C and 50 °C were 1.72±0.08 % ID/g and 1.44±0.14 % ID/g, respectively. In other bones, such as femur, skull, ribs and spine, the uptake of $^{68}$Ga-EDTMP prepared at 95 °C was also slightly higher than $^{68}$Ga-EDTMP formulations prepared at 20 °C and 50 °C, but statistical differences weren’t registered (figure 1).
All $^{68}$Ga-EDTMP formulations had lower uptake in soft organs and tissues as compared with bones. The uptake values in organs weren’t statistically significant for $^{68}$Ga-EDTMP formulations, except some terms (table 1). The highest uptake was observed in kidneys at 5 min p.i. due to urine excretion of phosphonates [7]. In blood only initial uptake of $^{68}$Ga-EDTMP prepared at 20 °C and 95 °C was 1.36±0.10 % ID/g and 1.33±0.08 % ID/g, respectively. In other organs and tissues the amounts of all $^{68}$Ga-EDTMP formulations didn’t exceed 1 % ID/g.

Femur/organs ratios were higher for $^{68}$Ga-EDTMP prepared at 50 °C and 95 °C than the corresponding data for $^{68}$Ga-EDTMP prepared at 20 °C, but statistical differences were observed only at some terms of study. For example, femur/blood ratios for $^{68}$Ga-EDTMP prepared at 50 °C and 95 °C reached 18.66±2.32 and 10.30±4.22, respectively, when for $^{68}$Ga-EDTMP prepared at 20 °C the maximal value was only 2.50±0.37. The highest femur/muscle ratios were for $^{68}$Ga-EDTMP formulation prepared at 95 °C: from 8.23±0.37 to 51.30±8.94. For $^{68}$Ga-EDTMP formulations prepared at 20 °C and 50 °C these ratios were 5.27-15.32 and 7.00-29.70, respectively.

4. Summary
It was shown that all $^{68}$Ga-EDTMP formulations prepared at different temperature had high bone uptake. However, the uptake of $^{68}$Ga-EDTMP prepared at 95 °C in bones, particularly in tibia, was higher when compared with $^{68}$Ga-EDTMP prepared at 20 °C. The amount of $^{68}$Ga-EDTMP prepared at 50 °C in bones...
didn’t have statistical differences with $^{68}$Ga-EDTMP prepared at 20 °C. The amounts of all $^{68}$Ga-EDTMP formulations in soft organs and tissues were low. In conclusion, heating a reaction mixture while radiolabeling had an influence on the biodistribution of $^{68}$Ga-EDTMP in bones.

**Table 1.** Biodistribution of $^{68}$Ga-EDTMP formulations in soft organs and tissues of Wistar rats at different time after intravenous injection (in % ID/g)

| Organ or tissue | 5 min | 1 h | 2 h | 3 h |
|----------------|-------|-----|-----|-----|
| Blood          | 1     |     |     |     |
|                | 1.36±0.10 | 0.77±0.16 | 0.63±0.06 | 0.57±0.04 |
|                | 2     |     |     |     |
|                | 0.93±0.04 | 0.64±0.11 | 0.38±0.06 | 0.09±0.01* |
|                | 3     |     |     |     |
|                | 1.33±0.08 | 0.82±0.07 | 0.59±0.17 | 0.21±0.07 |
| Lungs          | 1     |     |     |     |
|                | 0.70±0.08 | 0.32±0.04 | 0.31±0.03 | 0.30±0.03 |
|                | 2     |     |     |     |
|                | 0.42±0.03 | 0.29±0.06 | 0.27±0.01 | 0.20±0.03 |
|                | 3     |     |     |     |
|                | 0.74±0.14 | 0.34±0.04 | 0.30±0.03 | 0.31±0.03 |
| Liver          | 1     |     |     |     |
|                | 0.26±0.02 | 0.42±0.07 | 0.51±0.05 | 0.62±0.07 |
|                | 2     |     |     |     |
|                | 0.26±0.04 | 0.32±0.02 | 0.42±0.07 | 0.37±0.03* |
|                | 3     |     |     |     |
|                | 0.39±0.02 | 0.34±0.08 | 0.53±0.08 | 0.52±0.05 |
| Kidneys        | 1     |     |     |     |
|                | 1.71±0.46 | 0.36±0.05 | 0.28±0.03 | 0.21±0.03 |
|                | 2     |     |     |     |
|                | 1.17±0.19 | 0.32±0.03 | 0.25±0.04 | 0.20±0.05 |
|                | 3     |     |     |     |
|                | 1.56±0.35 | 0.56±0.22 | 0.29±0.06 | 0.25±0.05 |
| Heart          | 1     |     |     |     |
|                | 0.34±0.02 | 0.20±0.04 | 0.18±0.02 | 0.16±0.01 |
|                | 2     |     |     |     |
|                | 0.25±0.02 | 0.17±0.02 | 0.15±0.04 | 0.11±0.08 |
|                | 3     |     |     |     |
|                | 0.34±0.03 | 0.24±0.04 | 0.18±0.04 | 0.10±0.03 |
| Stomach        | 1     |     |     |     |
|                | 0.31±0.03 | 0.18±0.01 | 0.18±0.02 | 0.18±0.03 |
|                | 2     |     |     |     |
|                | 0.20±0.03 | 0.18±0.02 | 0.18±0.04 | 0.31±0.05 |
|                | 3     |     |     |     |
|                | 0.30±0.06 | 0.22±0.04 | 0.27±0.03 | 0.30±0.08 |
| Muscle         | 1     |     |     |     |
|                | 0.20±0.01 | 0.13±0.02 | 0.12±0.02 | 0.11±0.03 |
|                | 2     |     |     |     |
|                | 0.14±0.03 | 0.08±0.02 | 0.07±0.01 | 0.11±0.02 |
|                | 3     |     |     |     |
|                | 0.17±0.03 | 0.15±0.01 | 0.10±0.03 | 0.04±0.01 |

1 – $^{68}$Ga-EDTMP prepared at 20 °C
2 – $^{68}$Ga-EDTMP prepared at 50 °C
3 – $^{68}$Ga-EDTMP prepared at 95 °C

* – p < 0.05 as compared with $^{68}$Ga-EDTMP prepared at 20 °C

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