Biodistribution of phenylalanine labeled with gallium-68

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Abstract. Positron emission tomography (PET) is modern high sensitivity method of various tumor imaging. The synthesis of new radiopharmaceuticals based on amino acids and positron emitting radionuclide $^{68}$Ga for PET imaging is of great interest. This work is devoted to study the biodistribution of a new agent based on amino acid phenylalanine and $^{68}$Ga ($^{68}$Ga-phenylalanine) in Wistar rats with cholangioma RS-1 after intravenous administration. A comparative investigation of $^{68}$Ga-phenylalanine and $^{68}$GaCl₃ biodistribution was also carried out. It was shown that the highest uptake of $^{68}$Ga-phenylalanine was observed in blood, liver, femur and tumor. Tumor uptake of $^{68}$Ga-phenylalanine increased 3.5 times from 0.20 ± 0.03 % ID/g to 0.70 ± 0.10 % ID/g, whereas uptake of $^{68}$GaCl₃ decreased from 0.34 ± 0.07 % ID/g to 0.13 ± 0.04 % ID/g within 3 h. Blood uptake of $^{68}$Ga-phenylalanine reached 2.98 ± 0.31 % ID/g. In other organs and tissues the uptake of $^{68}$Ga-phenylalanine didn’t exceed 1 % ID/g. Kidneys and femur uptake of $^{68}$Ga-phenylalanine was lower as compared with $^{68}$GaCl₃, but in other organs the uptake of $^{68}$Ga-phenylalanine was similar or slightly higher when compared with $^{68}$GaCl₃.

1. Introduction

A high occurrence of cancer diseases is a serious medical problem. Early and precise disease detection is a key problem that determines further approaches and treatment potential. Nuclear medicine technologies play an important role in this problem solving.

Positron emission tomography (PET) is highly sensitive non-invasive diagnostic method that allows visualizing of metabolic abnormalities in organs and tissues long before the appearance of structural alterations. A wide spread of PET is impossible without high specific tumor-seeking radiopharmaceuticals.

Despite a large amount of synthesized radiopharmaceuticals, nowadays more than 90 % of PET surveys are performed with 2-deoxy-2-[$^{18}$F]fluoro-glucose ($^{18}$F-FDG). Automated $^{18}$F-FDG synthesis and local cyclotron availability for $^{18}$F production lead to high cost of survey and determine a broad application of PET [1]. PET with $^{18}$F-FDG is poorly suitable for visualization of tumors with low glycolytic rate such as neuroendocrine or brain tumors.

Thus, generator-produced radionuclides are of great interest. Gallium-68 ($^{68}$Ga) is a positron-emitting radionuclide with appropriate physical properties ($\beta^+ = 89\%$, $E_{\beta_{\text{max}}} = 1.9$ MeV) and short half-life ($T_{1/2} = 67.7$ min). Commercially available $^{68}$Ge/$^{68}$Ga generator is placed in hospitals, and $^{68}$Ga$^{5+}$ can be obtained during 12-18 months [2].
Aminoacids are promising carriers of radionuclides as they play an important role in metabolism, protein synthesis, signal and nerve impulses transmission between cells. Tumor cells are characterized by metabolic reprogramming, which is consisted in increasing of aminoacids consumption as compared with non-tumor cells [3]. Tumor cells are also characterized by upregulation of amino acid transporters such as LAT1 [4]. It is known that LAT1 is highly expressed in tumor cells of breast, prostate, lung, colorectal cancer, head and neck cancer, gliomas [5, 6].

Phenylalanine is essential amino acid, a source of tyrosine and protein synthesis. Tyrosine, in turn, is a precursor of dopamine, adrenaline and noradrenaline in nerve tissue and medullary layer of adrenal glands. Phenylalanine derivative, 3,4-dihydroxy-6-[18F]fluoro-L-phenylalanine (6-[18F]-DOPA), has been used for 30 years in PET to diagnose several nervous system disorders such as schizophrenia or Parkinson’s disease [7, 8]. Besides, 6-[18F]-DOPA can be used for diagnosis of neuroendocrine tumors (pheochromocytomas, pancreatic adenocarcinomas, etc.) due to neuroendocrine tumor cells consume and store the transported and decarboxylated amines in cytoplasmic neurosecretory granules [9].

Consequently, the development of new radiolabelled phenylalanine derivatives is of current interest of nuclear medicine. The aim of this work is to study the biodistribution of a promising agent for PET tumor imaging, 68Ga-phenylalanine, in Wistar rats with transplanted cholangioma RS-1.

2. Materials and methods

68Ga68Ge generator was obtained from Cyclotron Co., Ltd (Obninsk, Russia). Preparation of 68Ga-phenylalanine was the following: 10.0 mg of phenylalanine was dissolved in 2.0 ml of bidistilled water and adjusted with 3.0 M of HCl to pH of 2.0. Then 0.5 ml of 68GaCl3 in 0.05 M of HCl solution (3.7 MBq), stir for 5 min and adjusted with 0.1 M of sodium bicarbonate solution to pH of 5.6-6.0.

Radiochemical impurities were detected by paper chromatography in the same manner as described in [10]. The rate of 68Ga binding with phenylalanine was 92 % throughout the study.

All animal studies were carried out in female Wistar rats (140-160 g) with transplanted cholangioma RS-1. The transplantation of cholangioma RS-1 was performed as follows: the donor rat with tumor was sacrificed by cervical disruption, tumor tissue was removed, ground up and diluted in 0.9 % NaCl (1:3). Then this suspension (100 mg/rat in a volume of 0.1 ml) was injected subcutaneously into right flanks of Wistar rats. Ten days later, when the tumor volume reached 0.7-0.8 cm3, the rats were divided into 2 equal groups and used for biodistribution experiments. The animals of group 1 (n = 16) were administered intravenously with 0.37 MBq in 0.1 ml of 68Ga-phenylalanine. The animals of group 2 (n = 16) were intravenously injected with 0.37 MBq in 0.1 ml of 68GaCl3. At each time points after injection (5 min, 1, 2 and 3 h) four rats were sacrificed, the samples of tissues and organs were placed in tubes and weighed. The radioactivity was measured by automatic gamma counter. The amount of activity in organs and tissues was expressed as a percentage of the injected dose per gram of tissue (%ID/g). Also tumor/blood and tumor/muscle ratios were calculated. All the biodistribution studies 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 Student’s t test, and p<0.05 was considered statistically significant.

3. Results and discussion

The biodistribution of 68Ga-phenylalanine in tumor-bearing rats is represented in figure 1. The highest uptake of 68Ga-phenylalanine was observed in blood, liver, femur and tumor.

The initial tumor uptake of 68Ga-phenylalanine was 0.20 ± 0.03 % ID/g at 5 min post-injection (p.i.) and increased to 0.70 ± 0.10 % ID/g at 3 h p.i. In contrast, the uptake of 68GaCl3 decreased from 0.34 ± 0.07 % ID/g at 5 min to 0.13 ± 0.04 % ID/g at 3 h (figure 2).

In our previous investigations we studied the biodistribution of 68Ga-methionine [10], 68Ga-leucine [11], 68Ga-histidine and 68Ga-tryptophan [12]. The tumor uptake of 68Ga-methionine, 68Ga-histidine

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and \textsuperscript{68}Ga-tryptophan is similar to \textsuperscript{68}Ga-phenylalanine, increasing to maximal values to the end of the study [10, 12]. But the accumulation of \textsuperscript{68}Ga-leucine reduced from 0.79 ± 0.02 % ID/g to 0.29 ± 0.05 % ID/g throughout the study [11].

The peak blood uptake of \textsuperscript{68}Ga-phenylalanine and \textsuperscript{68}GaCl\textsubscript{3} was 2.98 ± 0.31 % ID/g and 3.64 ± 0.28 % ID/g at 5 min p.i., respectively. Further their amounts in blood decreased and became almost equal at 3 h p.i. High amounts of \textsuperscript{68}Ga-methionine, \textsuperscript{68}Ga-histidine and \textsuperscript{68}Ga-tryptophan in blood were observed [10, 12]. Only blood uptake of \textsuperscript{68}Ga-leucine (up to 1.40 ± 0.25 % ID/g) was lower [11].

\begin{figure}
\centering
\includegraphics[width=\textwidth]{biodistribution.png}
\caption{The biodistribution of \textsuperscript{68}Ga-phenylalanine in tumor-bearing Wistar rats (in %ID/g); SI – small intestine}
\end{figure}

The accumulation of \textsuperscript{68}Ga-phenylalanine in kidneys varied from 0.42 ± 0.07 to 0.55 ± 0.06 % ID/g, whereas kidneys uptake of \textsuperscript{68}GaCl\textsubscript{3} was 3.3-6.5 times higher and reached 3.57 ± 0.89 % ID/g at 5 min p.i. (figure 2). But at the next terms kidneys uptake of \textsuperscript{68}GaCl\textsubscript{3} reduced to 1.69 ± 0.24 % ID/g and stayed constant to the end of study.

In liver the amount of \textsuperscript{68}Ga-phenylalanine was 0.54-0.74 % ID/g. There are no statistical differences with \textsuperscript{68}GaCl\textsubscript{3} uptake in liver.

The brain uptake of \textsuperscript{68}Ga-phenylalanine was 3.4-4.5 higher as compared with \textsuperscript{68}GaCl\textsubscript{3} (p < 0.01). The peak amount of \textsuperscript{68}Ga-phenylalanine in brain was 0.118 ± 0.012 % ID/g at 5 min p.i., whereas the amount of \textsuperscript{68}GaCl\textsubscript{3} didn’t exceed 0.035 ± 0.008 % ID/g. It can be explained by high expression of L-type amino acid transporters LAT1 in endothelial cells of brain-blood barrier [13].

It is known that unbound \textsuperscript{68}Ga has high affinity to hydroxyapatite of bones [14]. For this reason the femur uptake of \textsuperscript{68}GaCl\textsubscript{3} reached 3.03 ± 0.62 % ID/g. The femur uptake of \textsuperscript{68}Ga-phenylalanine increased from 0.44 ± 0.04 to 0.93 ± 0.16 % ID/g that was lower than that of \textsuperscript{68}GaCl\textsubscript{3} (figure 2).
In other organs and tissues (lungs, heart, spleen, stomach, small intestine and muscle) the uptake of 
\(^{68}\text{Ga-phenylalanine}\) was similar or slightly higher when compared with \(^{68}\text{GaCl}_3\). The specific activity of \(^{68}\text{Ga-phenylalanine}\) in these organs didn’t exceed 1 % ID/g (figure 1).

* – p < 0,05 as compared with control group (\(^{68}\text{GaCl}_3\))

**Figure 2.** A comparative uptake of \(^{68}\text{Ga-phenylalanine}\) and \(^{68}\text{GaCl}_3\) in some organs of tumor-bearing Wistar rats (in %ID/g)

**Table 1.** Tumor/blood and tumor/muscle ratios of \(^{68}\text{Ga-phenylalanine}\) in soft organs and tissues of Wistar rats at different time after intravenous injection (in % ID/g)

|                        | Time after injection |
|------------------------|----------------------|
|                        | 5 min                | 1 h                  | 2 h                  | 3 h                  |
| Tumor/blood            | 0.07±0.01            | 0.26±0.04            | 0.37±0.07            | 0.61±0.15            |
| Tumor/muscle           | 1.10±0.12            | 2.99±0.48            | 3.99±0.60            | 6.27±1.55            |

Tumor/blood and tumor/muscle ratios for \(^{68}\text{Ga-phenylalanine}\) increased throughout the study (table 1). Thus, tumor/muscle ratios reached 6.27 ± 1.55 at 3 h p.i. Unfortunately, tumor/muscle ratios were less than 1 within the study. Also tumor/blood and tumor/muscle ratios for \(^{68}\text{Ga-phenylalanine}\) were higher than for \(^{68}\text{GaCl}_3\).

**4. Summary**

It was shown that tumor uptake of \(^{68}\text{Ga-phenylalanine}\) was higher than \(^{68}\text{GaCl}_3\) uptake. Moreover, the concentration of \(^{68}\text{Ga-phenylalanine}\) in tumor increased throughout the study. Sufficiently high uptake of \(^{68}\text{Ga-phenylalanine}\) in blood was observed. In other organs and tissues the uptake of \(^{68}\text{Ga-phenylalanine}\) was similar or slightly higher when compared with \(^{68}\text{GaCl}_3\) and didn’t exceed 1 % ID/g. Tumor/blood ratios were less than 1, whereas tumor/muscle ratios varied from 1.10 to 6.27.
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