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Preliminary Biological Evaluation of Leucine Labeled with Gallium-68—A Potential Agent for Tumor Imaging

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Abstract

Amino acids are important nutrients for proliferating tumor cells, so their transport is generally increased in many malignant tumor cells. Radiolabeled amino acids are of great interest as they can be alternative or complement tracers to the already well-established radiopharmaceuticals such as ^{18}F-FDG.

The purpose of this study was to synthesize and characterize a novel ^{68}Ga labeled leucine analog, ^{68}Ga-leucine, as a potential imaging agent for tumors which may not be amenable to imaging by ^{18}F-FDG PET. Biodistribution studies of ^{68}Ga-leucine were performed in Wistar rats with transplanted cholangioma RS-1 xenografts after intravenous injection.

^{68}Ga-leucine demonstrated high in vivo stability. Accumulation of ^{68}Ga-leucine at xenograft tumors was about 2-4 higher as compared with $^{68}GaCl_3$ and reached 0.79% ID/g. Among the soft tissue organs, only kidney had a relatively high uptake. The amount of radioactivity in other organs didn't exceed 1% ID/g. The results suggest that ^{68}Ga-leucine has the potential to be a new additional diagnostic tool for PET imaging of tumors.

Keywords: gallium-68, leucine, radiolabeled amino acids, positron emission tomography, tumor imaging.

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1. Introduction

Positron emission tomography (PET) has become an established method for medical research and clinical routine diagnostics because of its high sensitivity. The development and availability of new radiopharmaceuticals is one of the major conditions of the expansion of clinical PET. Nowadays a glucose analog, 2-^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG), is the most widely used as a PET tracer. Although ^{18}F-FDG has been successfully used in many cancers as a biomarker of glucose transport, it fails in diagnosis of

slowly growing tumors and in differentiation between tumor and such processes as inflammation, infection, reactive lymph nodes, tuberculosis and sarcoidosis [1, 2]. So the alternative tracers are of great interest.

Radiolabeled amino acids are expected to be promising PET agents due to their vital roles in many cellular processes including protein synthesis, energy metabolism, cell signaling, carbon sources for cell growth, and neurotransmission. Many tumor cells accumulate increased amount of amino acids as compared with normal cells, which is likely related to the increased expression of amino acids transporters in tumors [3]. Several ^{11}C- and ^{18}F-labeled amino acids such as ^{11}C-methionine and *O*-(2-^{18}F-fluoroethyl)-L-tyrosine have been used as PET tumor imaging agents in humans [4]. Leucine is one of the preferential amino acid required for proliferating tumor cells as a substrate for protein synthesis and is, therefore, of interest in molecular imaging of anabolic cancer processes.

Gallium-68 (^{68}Ga) is an emerging radionuclide for PET. ^{68}Ga is obtained from a ^{68}Ge/^{68}Ga generator system, which is simple in use and relatively inexpensive. Furthermore, ^{68}Ga offers appropriate decay properties ($T_{1/2}$ = 67.7 min, β^+ = 89%, $E_{\beta max}$ = 1.9 MeV) and the long physical $T_{1/2}$ of the parent radionuclide (270.8 d) allows the use of the generator for up to one year [5].

In this study, we evaluated the biodistribution of natural amino acid leucine labeled with ^{68}Ga in Wistar rats bearing cholangioma RS-1 tumor xenografts.

2. Materials and methods

Biodistribution studies were performed in Wistar rats weighing 140-160 g with tumor xenografts of cholangioma RS-1. To get a solid form of cholangioma RS-1 the donor rat with tumor was killed by cervical disruption, and the tumor tissue was isolated. Then the tumor tissue was ground up, diluted in physiological saline and implanted subcutaneously in rats. Biodistribution studies were performed after 8-10 days, when the tumor volume reached 0.7-0.8 cm^3.

32 Wistar rats with tumor xenografs were injected with 0.37 MBq of ^{68}Ga-leucine or $^{68}GaCl_3$ (n = 16 each tracer) in a volume of 0.1 ml through the tail vein. Animals were sacrificed at 5 min, 1, 3 and 5 h after injection. Four rats were used for each time points. The samples of tissues and organs were isolated, weighed and counted in automatic gamma counter "Wizard" version 2480 (PerkinElmer/Wallac, Finland). Data were calculated as a percentage of the injected dose per gram of tissue (%ID/g). All

the biodistribution studies were carried out in strict compliance with the national laws related to the conduct of animal experiments.

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 studies of ^{68}Ga-leucine are presented in Table 1. It was revealed that tumor uptake of ^{68}Ga-leucine was 0.79% ID/g at 5 min, 0.36% ID/g at 1 h, 0.32% ID/g at 3 h, and 0.29% ID/g at 5 h. These values were 2-4 times higher as compared with $^{68}GaCl_3$.

Amino acids are transported into cells by specific carrier-mediated transport system. Large neutral L-amino acids such as leucine are substrates of system L amino acid transporters. The level of one of these, LAT1, is overexpressed in many tumors [3]. For example, a new L-leucine analog, 5-[^{18}F]fluoroleucine is taken up by tumor cells via LAT1 transporter [6, 7]. One of the potential limitations of system L substrates is the ability of these transporters to mediate both influx and efflux of substrates, limiting the tumor-to-background ratios that can be achieved. It can explain a lack of radioactivity in tumor tissue after intravenous injection of ^{68}Ga-leucine.

The activity level in kidneys was the highest immediately after intravenous injection of ^{68}Ga-leucine (up to 4.60% ID/g). However, the uptake of radioactivity in kidneys was found to reduce with time, indicating that the excretion of injected activity was occurred through the urinary routes.

The radioactivity in the blood was as high as 1.40% ID/g at 5 min, but then it decreased to 0.58-0.63% ID/g and remained constant within 5 h. The levels of $^{68}GaCl_3$ were higher as compared with ^{68}Ga-leucine due to remarkable protein binding capacity of $^{68}Ga^{3+}$.

It is known that free ^{68}Ga has high affinity to hydroxyapatite and cortical matrix of bone [8]. Femur uptake of $^{68}GaCl_3$ reached 3.03% ID/g at 1 h after injection, whereas the highest activity of ^{68}Ga-leucine was 0.27% ID/g only. It is the evidence of high in vivo stability of ^{68}Ga-leucine.

The amounts of radioactivity in other organs such as lungs, liver, heart, spleen, stomach, small intestine, muscle and brain, didn't exceed 1% ID/g. Only uptake of ^{68}Ga-leucine in spleen reached 1.12% ID/g at 1 h.

Table 1: Specific amounts of radioactivity in organs and tissues of Wistar rats with transplanted cholangioma RS-1 at different time after intratumoral injection of ^{68}Ga-leucine and $^{68}GaCl_3$ (in % of injected dose per gram).

Organ/tissue	Time after injection			
	5 min	1 h	3 h	5 h
Blood	1.40±0.25* 3.64±0.28** p<0.001	0.61±0.03 2.43±0.23 p<0.001	0.58±0.11 1.06±0.17 p>0.05	0.63±0.03 0.78±0.09 p>0.1
Lungs	0.79±0.10 0.61±0.11 p>0.25	0.27±0.02 0.26±0.03 p>0.5	0.18±0.03 0.14±0.01 p>0.25	0.24±0.02 0.10±0.02 p<0.01
Liver	0.65±0.15 0.34±0.07 p>0.1	0.56±0.12 0.50±0.08 p>0.5	0.38±0.07 0.53±0.07 p>0.1	0.31±0.01 0.51±0.05 p<0.01
Kidney	4.60±0.43 3.57±0.89 p>0,25	1,03±0,12 1,69±0,24 p<0,05	0,60±0,06 1,67±0,17 p<0,002	0,64±0,03 2,05±0,34 p<0,01
Heart	0.41±0.06 0.35±0.05 p>0.25	0.18±0.02 0.09±0.02 p<0.02	0.16±0.03 0.03±0.01 p<0.01	0.17±0.02 0.03±0.01 p<0.001
Spleen	0.66±0.19 0.30±0.06 p>0.1	1.12±0.43 0.27±0.07 p>0.05	0.42±0.17 0.32±0.12 p>0.5	0.32±0.03 0.21±0.04 p>0.05
Stomach	0.47±0.12 0.30±0.07 p>0.25	0.14±0.01 0.14±0.02 p>0.5	0.08±0.01 0.07±0.01 p>0.5	0.10±0.01 0.06±0.01 p<0.05
Small intestine	0.31±0.05 0.33±0.08 p>0.5	0.13±0.01 0.21±0.04 p>0.1	0.10±0.02 0.10±0.01 p>0.5	0.15±0.01 0.09±0.01 p<0.001
Muscle	0.16±0.03 0.21±0.05 p>0.25	0.05±0.01 0.05±0.01 p>0.5	0.03±0.01 0.02±0.01 p>0.5	0.03±0.01 0.01±0.01 p>0.1
Femur	0.26±0.06 0.64±0.18 p>0.05	0.13±0.03 3.03±0.62 p<0.01	0.18±0.05 2.55±0.65 p<0.02	0.27±0.04 2.42±0.20 p<0.001
Brain	0.054±0.011 0.035±0.008 p>0.1	0.026±0.004 0.015±0.003 p>0.05	0.015±0.003 0.012±0.003 p>0.5	0.033±0.006 0.009±0.005 p<0.05
Tumor (cholangioma RS-1)	0.79±0.02 0.34±0.07 p<0.001	0.36±0.03 0.32±0.03 p>0.1	0.32±0.06 0.13±0.04 p<0.05	0.29±0.05 0.07±0.01 p<0.01
* – ^{68}Ga-leucine				
** – $^{68}GaCl_3$.				

4. Conclusion

Preliminary biodistribution studies in Wistar rats with cholangioma RS-1 xenografts revealed that ^{68}Ga-leucine demonstrated high in vivo stability. Tumor uptake of ^{68}Ga-leucine was about 2-4 times higher as compared with $^{68}GaCl_3$ and reached 0.79% ID/g. Among the soft tissue organs, only kidney had a relatively high uptake. The amount of radioactivity in other organs didn't exceed 1% ID/g. The results suggest that ^{68}Ga-leucine has the potential to be a new additional diagnostic tool for PET imaging of tumors.

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