

Preliminary biological evaluation of ^{99m}Tc -glucosamine as a potential radiotracer for tumor imaging

V K Tishchenko¹, V M Petriev^{1,2}, A.A. Mikhailovskaya¹, O.A. Smoryzanova¹, S A Ivanov¹ and A D Kaprin¹

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. The development of novel specific radiolabeled probes as targeted imaging agents for a more accurate detection of cancer has gained considerable interest. Glucose analogue ^{18}F -FDG is used worldwide for imaging of several cancer types. New glucose analogs radiolabeled with ^{99m}Tc could be a less-expensive and more accessible alternative for diagnosis using SPECT imaging. The complex of glucosamine and ^{99m}Tc were prepared, and its biodistribution was evaluated in solid Ehrlich carcinoma bearing mice. ^{99m}Tc -glucosamine was synthesized and radiolabeled with ^{99m}Tc with high yield and stability. Biodistribution studies showed that tumor uptake of ^{99m}Tc -glucosamine increased from 0.44 ± 0.06 %ID/g at 5 min postinjection (p.i.) to 0.54 ± 0.06 %ID/g and 0.64 ± 0.07 %ID/g at 1 h and 3 h p.i., respectively. The tumor/blood and tumor/muscle ratios also increased with time and reached 1.02 ± 0.12 and 1.88 ± 0.20 at 3 h p.i. ^{99m}Tc -glucosamine was rapidly removed from blood through the urinary system, so high amount of activity accumulated in kidneys. High uptake of ^{99m}Tc -glucosamine was also observed in two high-energy-dependent organs, heart and liver. In other organs such as lungs, spleen, stomach, small intestine and brain high uptake of ^{99m}Tc -glucosamine was only at 5 min after intravenous administration, but later the most part of activity was removed. In conclusion, we suggested that ^{99m}Tc -glucosamine would be a promising candidate for cancer SPECT imaging.

1. Introduction

An early and accurate noninvasive detection of cancer is an important factor in the treatment and prognosis of such patients. Radionuclide methods of tumor imaging can visualize the functional changes in organs and tissues before occurrence of anatomical changes and clinical signs of disease.

In comparison to normal tissues tumor growth is characterized by increased consumption of glucose, which is necessary to fulfill the biosynthetic demands associated with proliferation. ^{18}F -fluorodeoxyglucose (FDG) is routinely used in the clinic as a biomarker of metabolic activity for tumor diagnosis, staging and monitoring of treatment response. ^{18}F -FDG is transported into tumor cells by the glucose transmembrane transporters (GLUTs) and then is phosphorylated by hexokinases into FDG-6-phosphate [1]. FDG-6-phosphate cannot be metabolized further in the glycolytic pathway and accumulates within the cells [1].

Among the disadvantages of FDG is a short half-life (110 min) of fluorine. Also its production requires a quite expensive cyclotron and equipment for automated synthesis that lead to high costs of clinical doses of FDG [2]. Besides, FDG isn't a tumor specific agent and its uptake is described in a



number of non-neoplastic inflammatory lesions like sarcoidosis, tuberculosis, fungal infection and abscesses [3].

Hence, these limitations lead to the search for alternatives to FDG by utilizing gamma emitting radionuclides. Among them technetium-99m (^{99m}Tc) is of great interest due to its appropriate nuclear properties such as a half-life of 6.04 h and gamma emission energy of 0.140 MeV. It is produced from $^{99}\text{Mo}/^{99m}\text{Tc}$ generator. It is also the most widely used radionuclide in nuclear medicine: more than 90% of all scintigraphy and single photon emission computed tomography (SPECT) investigations are performed with ^{99m}Tc [2].

For these reasons the development of a tracer based on ^{99m}Tc that will mimic the biodistribution of FDG for tumor imaging is one of the most promising part of nuclear medicine. Glucosamine (2-amino-2-deoxy-D-glucose) is a highly attractive carrier for ^{99m}Tc . Literature data suggest that *N*-functionalized glucosamines show activity with GLUTs and hexokinases even when the functional group is large [4]. Besides, the amine group acts both as a potential coordination site and as a useful target for further functionalization [4]. So we synthesized glucosamine labeled with ^{99m}Tc and studied its biodistribution in tumor-bearing mice.

2. Methods and materials

Biodistribution experiments of ^{99m}Tc -glucosamine were performed on female mice weighting 18–25 g with subcutaneously transplanted Ehrlich carcinoma. For obtaining the solid Ehrlich carcinoma mice with ascites were used. Tumor cells ($2.5 \cdot 10^6$ in 0.1 ml) were implanted subcutaneously into the right hip of each mouse. The injection of ^{99m}Tc -glucosamine was performed a week later, when tumor volume reached 0.4–0.6 ml. All animals with tumors were injected intravenously into the tail vein with 0.37 MBq of ^{99m}Tc -glucosamine in a volume of 0.1 ml. Then animals were sacrificed by decapitation at different time intervals (5 min, 1 and 3 h) after injection. Four mice were used for each time point. Dissection began by drawing blood from the aorta. Then the samples of desired organs and tissues were collected, washed, weighted, and radioactivity was counted using automatic gamma counter. The obtained results were expressed as the percent of injected dose per gram of the organ (%ID/g). All the biodistribution studies were carried out in strict compliance with the national laws related to the conduct of animal experiments.

The obtained data were expressed as mean value \pm standard error of the mean ($M \pm m$). In addition, tumor/blood, tumor/kidneys and tumor/muscle ratios were calculated.

3. Results and discussion

The results of the biodistribution studies of ^{99m}Tc -glucosamine are summarized in figure 1. The highest uptake of radioactivity was observed in kidneys, heart, liver and tumor. In tumor the uptake of activity increased from 0.44 ± 0.06 %ID/g at 5 min postinjection (p.i.) to 0.54 ± 0.06 %ID/g at 1 h and 0.64 ± 0.07 %ID/g at 3 h p.i.

In works [5, 6] biological evaluation of ^{99m}Tc -ethylenedicysteine-glucosamine (^{99m}Tc -EC-DG) in tumor-bearing mice was investigated. In mice bearing lung cancer A549 tumor uptake of ^{99m}Tc -EC-DG was 0.79 ± 0.16 %ID/g, 0.42 ± 0.12 %ID/g, and 0.41 ± 0.16 %ID/g at 30 min, 2 and 4 h after intravenous administration, respectively [5]. In mesothelioma bearing mice the amount of ^{99m}Tc -EC-DG was also decreased from 0.47 ± 0.06 %ID/g at 30 min to 0.12 ± 0.01 %ID/g and 0.08 ± 0.00 %ID/g at 2 and 4 h, respectively [6]. These values were lower as compared with our results.

All organs had high uptake of ^{99m}Tc -glucosamine at 5 min after intravenous administration, but later the amounts of activity in the most of these organs were rapidly decreased. Thus, in blood the peak amount of ^{99m}Tc -glucosamine was 4.92 ± 0.30 %ID/g at 5 min p.i., decreasing approximately 4 fold to 1.24 ± 0.12 %ID/g at 1 h and 8 fold to 0.63 ± 0.06 %ID/g at 3 h p.i. Due to rapid excretion of radioactivity from blood and accumulation in tumor tumor/blood ratios were increased from 0.09 ± 0.01 at 5 min to 1.02 ± 0.12 at 3 h p.i., as shown in Table 1. Similar distribution was observed in liver, lungs, spleen, stomach, small intestine, brain, and femur. For example, in liver the highest activity was

3.42±0.34 %ID/g at 5 min p.i., but then it was only 0.87±0.30 %ID/g and 0.50±0.06 %ID/g at 1 and 3 h p.i., respectively.

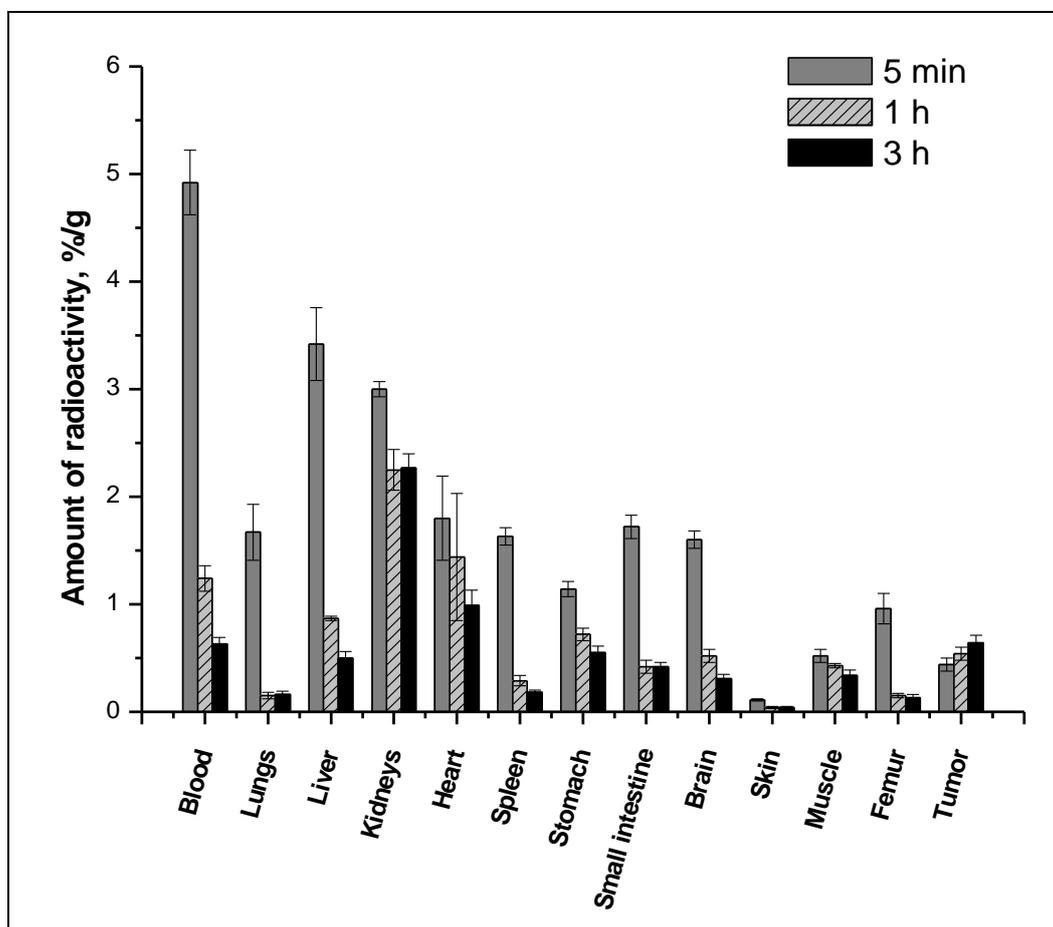


Figure 1. Biodistribution of ^{99m}Tc -glucosamine in tumor-bearing mice at different times after intravenous injection

In [4] the biodistribution of two deoxyglucose derivatives, ^{99m}Tc -(α,β)-2-deoxy-2-amino(ethylcarbamate)-D-glucose (ECB-DG) and ^{99m}Tc -(α,β)-2-deoxy-2-amino(1,2-dihydroxypropyl)-D-glucose (DHP-DG), was assessed on normal mice. Both complexes showed high liver, bowel, kidneys and lungs uptake. In liver amount of activity reached 21.85 %ID/g, in kidneys – 6.30 %ID/g, in lungs – 4.33 %ID/g. ^{99m}Tc -ECB-DG also accumulated in brain, and ^{99m}Tc -DHP-DG – in heart. High liver and kidneys uptake (up to 5.81 and 5.69 %ID/g, respectively) was also observed in lung cancer bearing mice after intravenous injection of ^{99m}Tc -EC-DG [5]. These data are in accordance with our results.

^{99m}Tc -glucosamine showed high renal accumulation throughout the study. It was probably due to renal route of activity excretion. Kidneys uptake of ^{99m}Tc -glucosamine was 3.00±0.07 %ID/g at 5 min p.i., then slightly decreased to 2.25-2.27 %ID/g. As shown in table 1, tumor/kidneys ratios were less 1, but raised from 0.15±0.02 to 0.28±0.04 during the study.

^{99m}Tc -glucosamine was slowly cleared from heart. Immediately after injection the amount of activity in heart reached 1.80±0.39 %ID/g. Later heart uptake of ^{99m}Tc -glucosamine decreased to 1.44±0.59 %ID/g at 1 h p.i. and 0.99±0.14 %ID/g at 3 h p.i. These values were higher as compared with tumor uptake, so ^{99m}Tc -glucosamine could be used as heart imaging agent.

Table 1. Tumor/blood, tumor/kidneys and tumor/muscle ratios in tumor-bearing mice after intravenous injection of ^{99m}Tc -glucosamine

Ratios	Time after injection		
	5 min	1 h	3 h
Tumor/blood	0.09±0.01	0.65±0.09	1.02±0.12
Tumor/kidneys	0.15±0.02	0.24 ±0.05	0.28±0.04
Tumor/muscle	0.85±0.15	1.26±0.13	1.88±0.20

Low ^{99m}Tc -glucosamine concentration was observed in skin (0.04-0.11 %ID/g) and muscle (0.34-0.52 %ID/g). Tumor/muscle ratio was 0.85±0.15 at 5 min p.i., but then ratios reached 1.26±0.13 and 1.88±0.20 at 1 h and 3 h, respectively.

4. Summary

In summary, a new glucose analogue, ^{99m}Tc -glucosamine, was synthesized and its biodistribution was assessed. Performed studies showed that tumor uptake of ^{99m}Tc -glucosamine increased throughout the study from 0.44 %ID/g to 0.64±0.07 %ID/g. The tumor/blood and tumor/muscle ratios also increased with time and exceeded 1 at 3 h p.i. ^{99m}Tc -glucosamine was rapidly removed from blood and other organs such as lungs, spleen, stomach, small intestine, brain, and femur through the urinary system, so high amount of activity accumulated in kidneys. High uptake of ^{99m}Tc -glucosamine was also observed in two high-energy-dependent organs, heart and liver. So we suggested that ^{99m}Tc -glucosamine would be a promising candidate for cancer SPECT imaging.

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