Biological behavior of a new 68Ga-labelled glucose derivative as a potential agent for tumor imaging

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Abstract. Glucose analogs and derivatives labeled with positron emitter 68Ga are considered to be a promising alternative to widely used radiotracer 18F-FDG for tumor PET imaging. In this study a biodistribution of a new glucose derivative labeled with 68Ga (68Ga-NODA-thioglucose) was investigated. All biodistribution studies were carried out in Balb/c mice with experimental model of tumor or aseptic inflammation. The tumor uptake of 68Ga-NODA-TG decreased throughout the study from 3.00±0.08 % ID/g to 1.06±0.04 %ID/g. The peak amount of 68Ga-NODA-TG in muscle with inflammation reached 4.33±0.12 % ID/g, decreasing to 0.23±0.08 % ID/g. In other organs and tissues the biodistribution of 68Ga-NODA-TG was similar in tumor-bearing mice and mice with aseptic inflammation. In conclusion, the obtained results suggest that 68Ga-NODA-TG has the potential for clinical application as a PET tracer.

1. Introduction

A significant feature of tumor cells is enhanced glucose uptake as well as their glycolytic rate, which can be up to 200 times greater as compared with normal cells [1]. This phenomenon is called Warburg effect and recognized as one of the hallmarks of cancer. Such shift of glucose metabolism to aerobic glycolysis for energy supply of tumor cells can serve as a promising target for diagnosis and therapy of cancer.

Nuclear medicine imaging plays an important role in diagnosis of cancer. Among them are single photon emission computed tomography (SPECT) and positron emission tomography (PET) that are used worldwide. PET is more sensitive, has better spatial resolution and offers quantification [2]. Nowadays 2-deoxy-2-[18F]fluoro-glucose (18F-FDG) remains the basic radiopharmaceutical for PET imaging. But the application of 18F-FDG is limited due to the need of a near cyclotron for producing 18F isotope and high cost [3]. Besides, some tumors have negligible 18F-FDG accumulation due to their low metabolic activity [4].

The generator produced positron emitting radionuclide 68Ga can be an alternative to 18F. 68Ga has appropriate decay characteristics for PET imaging: T1/2 = 68 min, β+ 89%, Emax = 1.9 MeV [5]. It can be obtained in 68Ga3+ cation form from commercially available generator 68Ge/68Ga. 68Ga3+ cation can form stable complexes with many ligands containing oxygen and nitrogen as donor atoms [5]. Glucose analogs and its derivatives are the suitable carriers for radionuclide delivery to cancer lesions. There are many glucose derivatives labeled with 99mTc, 111In, 18F, 64Cu for SPECT and PET tumor imaging, but none of them has been approved for clinical use yet [1, 2]. We developed new glucose derivative based
on thioglucose conjugated with NODA and labeled with $^{68}$Ga. The aim of this study was to evaluate the biodistribution of new agent in mice with tumor or inflammation.

2. Materials and methods

The labeled compound ($^{68}$Ga-NODA-thioglucose, or $^{68}$Ga-NODA-TG) was prepared as follows: 0.5 ml of deionized water was added to lyophilizate of NODA-TG and the mixture was stirred until precipitate was completely dissolved. Then 0.5 ml of 0.2 M acetate buffer with pH 4.6 was added and mixed. Then into the vial with lyophilizate 37 MBq (1.0 mCi) of gallium chloride $^{68}$GaCl$_3$ in 0.5 ml of 0.05 M HCl was added. The reaction mixture was mixed for 10 min at room temperature, brought up to a volume 2.0 ml by deionized water, and filtered through a 0.22 µm membrane filter.

Quality control of $^{68}$Ga-NODA-TG was performed as described in [6]. Radiochemical impurities in the $^{68}$Ga-NODA-TG compound did not exceed 5.0%.

All biodistribution studies were carried out in Balb/c mice with average weight of 15-20 g. All animals were divided into 2 groups (n = 16 for each group). The first group was undergone a tumor transplantation. Colon adenocarcinoma was chosen as a model of a malignant tumor. The procedure of transplantation is described in detail in [7]. When the tumor volume reached 0.5 cm$^3$, the mice were used for biodistribution experiments.

The second group of animals had aseptic inflammation. The experimental model of aseptic inflammation was induced by intramuscular injection (into the femoral muscle) of turpentine in a volume of 0.05 mL/mouse. During the next two days, aseptic inflammation was developed. Its presence in animals was estimated by palpation, as well as visually by behavioral responses and the presence of edema and hyperemia at the injection site.

Two groups of animals were injected intravenously with 0.18-0.37 MBq of $^{68}$Ga-NODA-TG in a volume of 0.1 ml. Animals were sacrificed at 5 min, 1, 2 and 3 h after injection. Four mice were used for each time points. The samples of tissues and organs were isolated, placed in plastic tubes and weighed. The radioactivity was measured by automatic gamma counter. The uptake was expressed 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 of biodistribution data for each group of mice were expressed as mean value and standard error of the mean (M ± SEM). Tumor/organs and muscle tissue with inflammation/organs ratios were calculated.

3. Results and discussion

The uptake of $^{68}$Ga-NODA-TG in mice with colon adenocarcinoma or aseptic inflammation is represented in figure 1. The initial tumor uptake of $^{68}$Ga-NODA-TG was 3.00±0.08 % ID/g. Later the uptake decreased to 1.48±0.09, 1.34±0.22 and 1.06±0.04 % ID/g at 1, 2 and 3 h post-injection (p.i.), respectively. In muscle tissue with inflammation the maximal uptake of $^{68}$Ga-NODA-TG reached 4.33±0.12 % ID/g at 5 min p.i. Then it decreased more than 5 fold to 0.80±0.17 % ID/g at 1 h p.i. At 2 and 3 h p.i. the uptake of $^{68}$Ga-NODA-TG was 0.24±0.01 % ID/g and 0.23±0.08 % ID/g, respectively. It is noteworthy that the uptake of $^{68}$Ga-NODA-TG in muscle tissue with inflammation was 2.7-5 times higher as compared with intact muscle. The highest muscle uptake was 1.61±0.16 % ID/g at 5 min p.i., dropped to 0.08-0.16 % ID/g throughout the study.

It is crucial that tumor uptake of $^{68}$Ga-NODA-TG was higher than other $^{68}$Ga-containing glucose derivatives, $^{68}$Ga-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-2-deoxy-D-glucosamine ($^{68}$Ga-DOTA-DG) and $^{68}$Ga-ethylenedicysteineglucosamine ($^{68}$Ga-ECG). The amount of $^{68}$Ga-ECG in rats with transplanted mesothelioma increased from 0.70 % ID/g at 15 min p.i. to 0.92 % ID/g at 1 h p.i. [8]. In mice with human epithelial carcinoma A431, the maximal tumor uptake of $^{68}$Ga-DOTA-DG was 2.38 % ID/g at 10 min p.i., decreased to 0.39 % ID/g at 1 h p.i. [9]. When compared with $^{68}$Ga-NODA-aminoglucose, its peak uptake in colon adenocarcinoma was 4.88 % ID/g at 5 min p.i. Then it decreased to 1.57, 0.54 and 0.52 % ID/g at 1, 2 and 3 h p.i., respectively [7]. $^{68}$Ga-NODA-aminoglucose was also accumulated (up to 6.31 % ID/g) in muscle with aseptic inflammation [10].
Figure 1. The uptake of $^{68}$Ga-NODA-TG in organs and tissues of Balb/c mice with colon adenocarcinoma (A) or aseptic inflammation (B) at different time after intravenous injection.

*SI – small intestine

In all soft organs and tissues of both groups of mice the peak uptake of $^{68}$Ga-NODA-TG was observed at 5 min p.i. At the next terms the amount of $^{68}$Ga-NODA-TG decreased many times.

The highest uptake of $^{68}$Ga-NODA-TG was registered in kidneys. The amount of $^{68}$Ga-NODA-TG in kidneys of tumor-bearing mice was 6.32-15.96 % ID/g (figure 1). In mice with aseptic inflammation kidneys uptake of $^{68}$Ga-NODA-TG was 1.97-16.95 % ID/g.

$^{68}$Ga-NODA-TG did not accumulate in high amounts in brain unlike $^{18}$F-FDG [8, 9]. The brain uptake of $^{68}$Ga-NODA-TG did not exceed 0.28 or 0.26 % ID/g in tumor-bearing mice and mice with inflammation, respectively, whereas the concentration of $^{18}$F-FDG in brain can rise up to 5.81 % ID/g [9].

Tumor/organs and muscle with inflammation/organs ratios increased throughout the study, as shown in figure 2. The tumor uptake of $^{68}$Ga-NODA-TG was higher than in most organs and tissues, except blood and kidneys. Tumor/blood and tumor/kidneys ratios were 0.42-0.58 and 0.17-0.20, respectively,
whereas tumor/muscle ratios reached 20.82. In contrast, tumor/blood ratio for $^{68}$Ga-NODA-aminoglucose was 3.38 already at 1 h p.i. and increased to 12.78 at 3 h p.i. [7].

Muscle with inflammation/blood ratios raised from 0.65 to 2.57 that was higher as compared with tumor/blood ratios. Muscle with inflammation/intact muscle ratios varied from 2.76 to 6.53 within the study. Only muscle with inflammation/kidneys ratios were less 1 (figure 2).

4. Summary

It was established that new glucose derivative, $^{68}$Ga-NODA-TG, accumulated in tumor and muscle tissue with inflammation, where its uptake was higher than in the most of soft organs and tissues. The highest uptake of $^{68}$Ga-NODA-TG was registered in kidneys. In other organs peak uptake of $^{68}$Ga-NODA-TG was observed only at 5 min p.i., decreasing significantly towards the end of study. In conclusion, the obtained results suggest that $^{68}$Ga-NODA-TG has the potential for clinical application as a PET tracer.

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