ISSN 1068-3356, Bulletin of the Lebedev Physics Institute, 2020, Vol. 47, No. 11, pp. 339–344. © Allerton Press, Inc., 2020.
Russian Text © The Author(s), 2020, published in Kratkie Soobshcheniya po Fizike, 2020, Vol. 47, No. 11, pp. 19–27.

Behavioral Features of Gallium-68 Radionuclide Incorporated in Glucose Derivatives in Laboratory Animals

V. M. Petriev[a,b,*], V. K. Tishchenko[a,b], E. D. Stepchenkova[a], O. V. Fedorov[c], and A.A. Fronya[b,d]

[a] *Tsyb Medical Radiological Research Center, Branch of the National Medical Research Radiological Center of the Ministry of Health of the Russian Federation, Obninsk, Kaluga oblast, 249036 Russia*

[b] *National Research Nuclear University "MEPhI," Moscow, 115409 Russia*

[c] *SC "Pharm-Sintez," Moscow, 111024 Russia*

[d] *Lebedev Physical Institute, Russian Academy of Sciences, Moscow, 119991 Russia*

***** *e-mail: petriev@mrrc.obninsk.ru*

Received September 5, 2020; revised September 15, 2020; accepted September 16, 2020

Abstract—This work is devoted to a comparative study of pharmacokinetic properties of complex compounds labeled with gallium-68 aminoglucose (^{68}Ga-NODA-AG) or thioglucose (^{68}Ga-NODA-TG) in BALB/c mice after intravenous administration. Statistical significance of activity accumulation in most organs and tissues was observed only at 5 min post-injection. At the following terms, these values didn't have any statistical significance in most organs and tissues. Activity was eliminated from organs and tissues with high rate. The biological and effective half-lives of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG from organs and tissues were less than 1 hour.

Keywords: gallium-68, glucose derivatives, aminoglucose, thioglucose, biological half-life, effective half-life

DOI: 10.3103/S1068335620110081

1. INTRODUCTION

The implementation of the positron emission tomography (PET) method to routine clinical practice stimulated the development of new radiopharmaceuticals for diagnostics of oncological diseases. Nevertheless, [^{18}F]-fluoro-2-deoxy-2-D-glucose (^{18}F-FDG) remains in high demand. Its applicability is caused by nonspecific accumulation in tumorous cells due to enhanced glucose absorption during malignization [1]. After entering tumor cell using glucose transporters (GLUTs), ^{18}F-FDG is phosphorylated by hexokinase enzyme and is transformed to ^{18}F-FDG-6-phosphate. Due to the ^{18}F atom, ^{18}F-FDG-6-phosphate is not undergone to further metabolic transformations and accumulated in cancer cells allowing tumor visualization.

The main disadvantage of ^{18}F-FDG is the requirement of using a cyclotron to produce ^{18}F which ought to be located near a PET center, as well as other expensive equipment for automated synthesis of ^{18}F-FDG [2]. Ultimately these factors increase the cost of procedure and thereby educe its availability to the population.

As an alternative to ^{18}F, the generator-produced radionuclide gallium-68 ($T_{1/2}$ = 68 min, β^+ = 89%, $E^+_{\beta\,max}$ = 1.9 MeV) is proposed [3]. An improvement of the ^{68}Ge/^{68}Ga generator, which makes possible to obtain $^{68}Ga^{3+}$ in the ionic form during elution, and the start of its commercial production stimulated the development of new compounds based on ^{68}Ga [3—7]. Glucose analogues or its derivatives can serve as vector molecules, that can deliver radioactive label exactly to tumor cells. Over the past two decades, a large number of compounds based on glucose analogues labeled with ^{99m}Tc, ^{111}In, ^{18}F, ^{64}Cu radionuclides have been obtained, and their applicability of tumor metabolism visualization has been shown [8].

The aim of this study was to compare pharmacokinetic properties of two new compounds based on aminoglucose and thioglucose labeled with ^{68}Ga in intact laboratory animals.

Table 1. Half-lives of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG in organs and tissues of intact mice BALB/c

No.	Organ, tissue	Half-lives, hours			
		T_{biol}		T_{eff}	
		^{68}Ga-NODA-AG	^{68}Ga-NODA-TG	^{68}Ga-NODA-AG	^{68}Ga-NODA-TG
1	Blood	0.21	0.26	0.18	0.21
2	Lungs	0.24	0.26	0.20	0.21
3	Liver	0.61	–	0.40	–
4	Kidneys	0.21	0.21	0.18	0.18
5	Heart	0.25	0.22	0.20	0.18
6	Spleen	0.17	0.78	0.15	0.46
7	Stomach	0.21	0.17	0.18	0.15
8	Small intestine	0.27	0.22	0.22	0.18
9	Brain	0.35	0.24	0.27	0.20
10	Muscle tissue	0.25	0.20	0.20	0.17
11	Femur	0.23	0.27	0.19	0.22
12	Skin	0.23	0.26	0.19	0.21

2. MATERIALS AND METHODS

The labeled compound (^{68}Ga-NODA-AG) was prepared by administration of gallium chloride ($^{68}GaCl_3$) into a vial with lyophilizate. For this, 0.5 ml of deionized water was added to NODA-AG lyophilizate and stirred until precipitate was completely dissolved, 0.5 ml of 0.2 M acetate buffer with pH 4.6 was added and mixed. 37 MBq (1.0 mCi) of $^{68}GaCl_3$ in 0.5 ml of 0.05 M HCl. Then 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 filter with a pore size of 0.22 μm.

For the quantitative determination of ^{68}Ga bound to NODA-AG, free ^{68}Ga (not bound to NODA-AG), and hydrolyzed ^{68}Ga paper chromatography was used. Whatman-1 (Sigma-Aldrich) was used as a stationary phase. As mobile phases, 1.0 M acetate solution and 0.05% citric acid solution were chosen. When eluting with an 1.0 M sodium acetate solution, ^{68}Ga bound with NODA-AG moved with the eluent front (R_f = 0.85—0.95), free ^{68}Ga remained at the start (R_f = 0). When eluting with 0.05% citric acid solution, hydrolyzed ^{68}Ga slightly shifted from the start (R_f = 0.05—0.10) when free ^{68}Ga and ^{68}Ga bound with NODA-AG ascended with the eluent front (R_f = 0.85—0.95).Hydrolyzed ^{68}Ga, free and NODA-AG-bound ^{68}Ga were quantitatively deterined by calculating the results of radiometry of chromatographic paper strips. Radiometry was performed using an automated Wizard gamma counter version 2480 (Perkin-Elmer/Wallac, Inc. (Finland)).

Obtained radiopharmaceuticals were intended for intravenous injections.

Radiochemical impurities in the ^{68}Ga-NODA-AG compound did not exceed 5.0%.

Synthesis and analysis of radiochemical impurities of ^{68}Ga-NODA-TG is similar to ^{68}Ga-NODA-AG. Radiochemical impurities in the ^{68}Ga-NODA-TG compound did not exceed 5.0%.

The pharmacokinetic study of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG experimental samples was performed on BALB/c intact mice with weight 15—20 g. Animals were divided into two equal groups of 16 mice in each. Animal of the first and second groups were injected intravenously (into vein) of ^{68}Ga-NODA-AG or ^{68}Ga-NODA-TG, respectively, with a dose of 0.37 MBq and in a volume of 0.1 ml.

At 5 min, 1, 2, and 3 hours after administration, four animals for each time point were put to euthanasia by decapitation (under narcosis) to obtain a blood sample. Organ and tissues were also collected into plastic tubes, weighted on electronic balance, and radiometry was performed using an automated gamma counter "Wizard" version 2480 (PerkinElmer/Wallac Finland). The data were expressed as a pecentage of the injected dose per gram of tissue.

All applicable international, national, and institutional principles for animal care were followed.

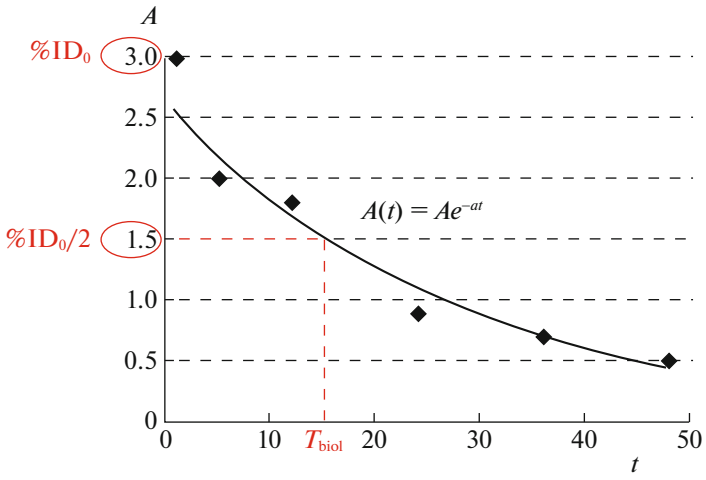

Fig. 1. Time dependence of the radiopharmaceutical concentration in organ *i*.

When statistical processing the results of radiometry, arithmetic mean values (M) and standard errors of the mean (±m) were determined using Excel program (Microsoft).

The data of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG biological half lives were calculated based on obtaining exponential curve and assuming that the excretion of activity from organs and tissues is performed exponentialy (Fig. 1).

If the slope A and the rate constant a are matched as accurately as possible to plot the curve, the point with the half of activity will correspond to the biological half-life T_{biol}.

Thus, it is easy to express the biological half-life T_{biol} from formula $A(t) = A \cdot e^{-at}$,

$$T_{biol} = \frac{1}{a} \cdot \ln\left(\frac{2A}{\%ID_0}\right),$$

where T_{biol} is the biological half-life of a compound in organ or tissue, h; A is the slope at exponential function; a is the rate constant, h^{-1}; $\%ID_0$ is the initial percent of injected dose per organ or tissue, %/organ.

The effective half-lives were calculated using the formula

$$T_{eff} = \frac{T_{biol} \cdot T_{1/2}}{T_{biol} + T_{1/2}},$$

where T_{eff} is the effective half-life, h; T_{biol} is the biological half-life for in organ or tissue, h; $T_{1/2}$ is the physical half-life of radionuclide, h.

Experimental data were approximated and biological half-lives were calculated using OriginPro 2019b program.

3. RESULTS AND DISCUSSION

According to the obtained results, the highest uptake of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG was detected in kidneys, and the lowest uptake was registered in brain. At the same time, peak levels of activity in most organs were observed at 5 min post-injection (p.i.) of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG (Fig. 2).

In kidneys the initial level of ^{68}Ga-NODA-AG was 24.82%/g; however, this value decreased eightfold to 2.99%ID/g ($p < 0.001$), slightly changing (1.79–2.06%ID/g) at subsequent terms ($p > 0.05$). The accumulation of ^{68}Ga-NODA-TG in kidneys did not exceed 14.04%ID/g in 5 min and 2.11–2.59%ID/g at 1–3 h p.i. It is important that the accumulation values of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG in kidneys were higher than in other organs and tissues at all times. This is more likely due to the elimination of labeled compounds through the urinary system. Nevertheless, ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG were characterized by the same half lives of activity from the kidneys ($T_{biol} = 0.21$ h, $T_{eff} = 0.18$).

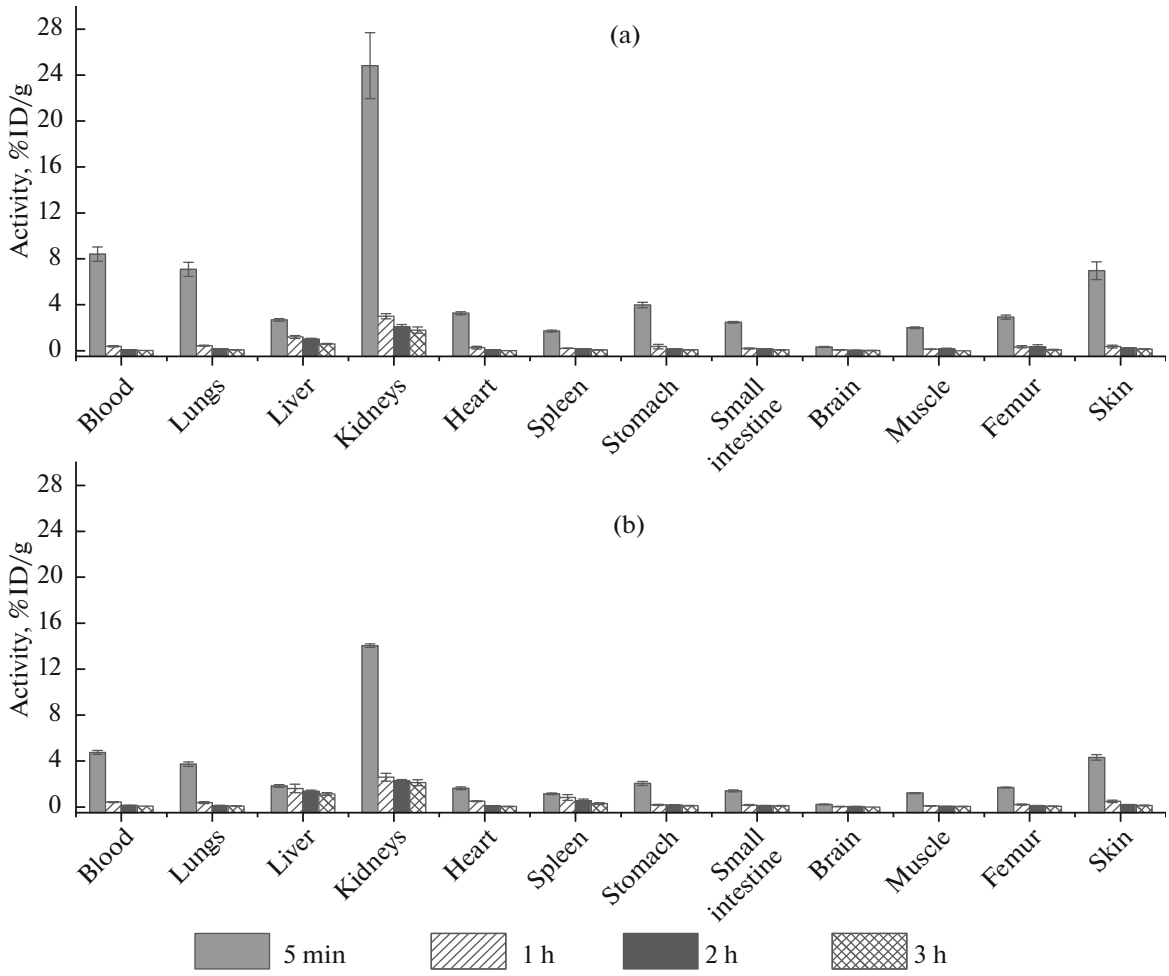

Fig. 2. The amount of (a) ^{68}Ga-NODA-AG and (b) ^{68}Ga-NODA-TG in organs and tissues of intact mice BALB/c at different times after intravenous administration.

In blood the concentration of ^{68}Ga-NODA-AG was higher than ^{68}Ga-NODA-TG at 5 min p.i. (8.41%ID/g and 4.75%ID/g, respectively). At 1 h p.i. the concentration of ^{68}Ga-NODA-TG (0.02–0.39%ID/g) was higher in comparison with ^{68}Ga-NODA-AG (0.08–0.43%ID/g) due to a higher removal rate of activity from blood of ^{68}Ga-NODA-AG than that of ^{68}Ga-NODA-TG.

A similar tendency in the distribution of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG was observed in other organs and tissues. At 5 min p.i., the concentration of ^{68}Ga-NODA-TG was lower in comparison with ^{68}Ga-NODA-AG; however later the concentration of ^{68}Ga-NODA-AG ^{68}Ga-NODA-AG in organs and tissues was slightly lower than that of ^{68}Ga-NODA-TG (Fig. 2).

Currently, only two compounds based on glucose and ^{68}Ga derivatives are known: ^{68}Ga-1,4,7,10 tetraazacyclododecane-1,4,7,10- tetraacetic acid-2-deoxy-D-glucosamine (^{68}Ga-DOTA-DG) and ^{68}Ga-ethylene dicysteine-glucosamine (^{68}Ga-ECG) [9, 10]. The study of their biodistribution was carried out in animals with transplanted unlike ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG, the amount of ^{68}Ga-ECG in blood decreased insignificantly [10]. The level of ^{68}Ga-ECG in kidneys was also low [10].

The concentration of ^{68}Ga-DOTA-DG in blood decreased rapidly, as well as those of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG [9]. In kidneys, the accumulation of ^{68}Ga-DOTA-DG was high through the study and was similar to ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG [9]. An increased accumulation of activity and long-term retention in kidneys was also observed after intravenous administration of ^{99m}Tc glucosamine [11].

The minimum accumulation ^{of} ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG was registered in the brain. The ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG concentrations were 0.02—0.32%/g and 0.03–0.22%ID/g, respectively (Fig. 2). Thus, ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG were characterized by minimal accumulation in the brain, which distinguished them favorably from ^{18}F-FDG which concentration in brain can reach 2.36–5.81%ID/g [9, 10]. The low concentration of ^{68}Ga-NODA-AG in brain (up to 0.43%ID/g) was previously observed in mice with subcutaneously transplanted colon adenocarcinoma [12]. Furthermore, a high amount of ^{18}F-FDG is also registered in the heart [9, 10], whereas accumulation of ^{68}Ga-containing glucose derivative in the heart was very low. For example, the concentrations were 0.01–3.26%ID/g and 0.08–1.62%ID/g for ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG, respectively.

Both compounds ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG were characterized by high clearances from organs and tissues. Interestingly that removal of ^{68}Ga-NODA-AG was most rapid from the spleen (T_{biol} = 0.17 h, T_{eff} = 0.15 h); while the half-life of ^{68}Ga-NODA-TG from the spleen was the highest (T_{biol} = 0.78 h, T_{eff} = 0.46 h). ^{68}Ga-NODA-AG was removed faster than ^{68}Ga-NODA-TG from lungs, femur and skin. In turn, ^{68}Ga-NODA-TG was characterized by enhanced excretion from the heart, stomach, small intestine, brain and muscle tissue in comparison with ^{68}Ga-NODA-AG.

The elimination of ^{68}Ga-NODA-AG from liver was slightly slower as compared with other organs (T_{biol} = 0.61 h, T_{eff} = 0.40 h). The concentration of ^{68}Ga-NODA-TG in the liver decreased insignificantly: from 1.83 at 5 min to 1.13%ID/g at 3 h p.i., where was no twofold decrease of ^{68}Ga-NODA-TG in the liver thoughout the study, and the excretion was not described by the exponential function. Therefore, the calculation of ^{68}Ga-NODA-TG half-life from the liver was impossible.

4. CONCLUSIONS

Thus, after a single intravenous administration of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG to intact mice BALB/c, the activity was rapidly cleared from organs and tissues. The biological and effective half-lives of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG did not exceed 1 hour. The highest concentration of activity was observed only at 5 min p.i. of ^{68}Ga-NODA-AG and ^{68}Ga-NODA-TG. The highest accumulation of activity was registered in kidneys.

FUNDING

This study was supported by the Ministry of Education and Science of the Russian Federation (agreement no. 075-02-2018-097, November 26, 2018); Unique project identifier is RFMEFI57518X0174.

REFERENCES

1. C. Marbaniang and L. Kma, "Dysregulation of Glucose Metabolism by Oncogenes and Tumor Suppressors in Cancer Cells," Asian Pac. J. Cancer Prev. **19**, 2377 (2018).
2. V. M. Petriev, V. K. Tishchenko, and R. N. Krasikova, "^{18}F-FDG and Other Labeled Glucose Derivatives for Use in Radionuclide Diagnosis of Oncological Diseases (Review)," Pharm. Chem. J. **50**, 209 (2016). https://doi.org/10.1007/s11094-016-1425-y
3. I. Velikyan, "Prospective of ^{68}Ga-Radiopharmaceutical Development," Theranostics **4**, 47 (2014).
4. V. K. Tishchenko, V. M. Petriev, I. N. Zavestovskaya, S. A. Ivanov, and A. D. Kaprin, "Osteotropic Radiopharmaceutical Preparations Based on Phosphonic Acids and ^{68}Ga (Review)," Radiation and Risk **29**, 102 (2020).
5. V. M. Petriev, V. K. Tishchenko, E. D. Stepchenkova, I. N. Zavestovskaya, P. V. Shegai, S. A. Ivanov, and A. D. Kaprin, "Effect of Gallium Carrier in ^{68}Ga-Ethylenediaminetetrakis (Methylene Phosphonic Acid) on its Behavior in Laboratory Animals," Bull. Lebedev Phys. Inst. **46**, 319 (2019). https://doi.org/10.3103/S1068335619100051
6. V. K. Tishchenko, V. M. Petriev, E. D. Stepchenkova, V. Yu. Timoshenko, A. A. Postnov, and I. N. Zavestovskaya, "Experimental Study of the Biodistribution of New Bone-Seeking Compounds Based on Phosphonic Acids and Gallium-68," Bull. Exp. Biol. Med. **168**, 777 (2020).
7. V. M. Petriev, V. K. Tishchenko, O. A. Smoryzanova, I. N. Zavestovskaya, and A. A. Postnov, "Complex Compounds of Rhenium-188 and Gallium-68 Radionuclides and Their Behavior in the Organism of Laboratory Animals," Bull. Lebedev Phys. Inst. **46**, 58 (2019). https://doi.org/10.3103/S1068335619020052

8. H. Feng, X. Wang, J. Chen, J. Cui, T. Gao, Y. Gao, and W. Zeng, "Nuclear Imaging of Glucose Metabolism: Beyond ^{18}F-FDG," Contrast Media Mol. Imaging **2019**, 7954854 (2019).
9. Z. Yang, C. Xiong, R. Zhang, H. Zhu, and C. Li, "Synthesis and Evaluation of ^{68}Ga-Labeled DOTA-2-deoxy-D-glucosamine as a Potential Radiotracer in μPET Imaging," Am. J. Nucl. Med. Mol. Imaging **2**, 499 (2012).
10. Y. H. Zhang, J. Bryant, F. L. Kong, D. F. Yu, R. Mendez, E. E. Kim, and D. J. Yang, "Molecular Imaging of Mesothelioma with ^{99m}Tc-ECG and ^{68}Ga-ECG," J. Biomed. Biotechnol. 2012**:**232863.
11. V. K. Tishchenko, V. M. Petriev, A. A. Mikhailovskaya, O. A. Smoryzanova, S. A. Ivanov, and A. D. Kaprin, "Preliminary Biological Evaluation of ^{99m}Tc-Glucosamine as a Potential Radiotracer for Tumor Imaging," IOP Conf. Ser.: J. Phys.: Conf. Ser. **1439**, 012033 (2020).
12. V. K. Tishchenko, V. M. Petriev, A. A. Mikhailovskaya, E. D. Stepchenkova, and I. N. Zavestovskaya, "Pharmacokinetic Properties of a New Potential Tumor Imaging Agent Based on Glucose Derivative and Gallium-68," in *4th International Symposium and School for Young Scientists on "Physics, Engineering and Technologies for Biomedicine": Book of Abstracts* (MEPhI, Moscow, 2019), pp. 62—63.

Translated by A. Kazantsev