Development and Study of ^{99m}Tc-1-Thio-D-glucose for Visualization of Malignant Tumors

R. Zeltchan[1,a)], A. Medvedeva[1], I. Sinilkin[1], O. Bragina[1], V. Chernov[1,2], E. Stasyuk[2], A. Rogov[2], E. Il'ina[2], and V. Skuridin[2]

[1] *Cancer Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, 634050 Russia*
[2] *National Research Tomsk Polytechnic University, Tomsk, 634050 Russia*

[a)] Corresponding author: r.zelchan@yandex.ru

Abstract. The preclinical studies of ^{99m}Tc-1-Thio-D-glucose, a new tumor-seeking agent based on technetium-99m-labeled glucose derivative, were conducted, and the feasibility of using this radiopharmaceutical for tumor visualization was studied. The preclinical studies were carried out strictly in accordance with the local legislation and were regulated by the generally accepted research standards. ^{99m}Tc-1-Thio-D-glucose was found to have optimal pharmacokinetic and physico-chemical properties for diagnostic imaging and was proved to belong to the low-toxic substances. The potential utility of ^{99m}Tc-1-thio-D-glucose for tumor imaging was studied in vitro and in vivo models. The present study demonstrated that ^{99m}Tc-1-Thio-D-glucose is a prospective radiopharmaceutical for cancer visualization.

INTRODUCTION

Early cancer detection is known to be a key element in increasing survival of cancer patients. Cancer is the second leading cause of death in Russia. The mortality rate has increased by 13.8% over the last 10 years. Approximately 480,000 new cancer cases are diagnosed each year in Russia. Unfortunately, 60% of cancer patients are diagnosed in advanced stages. The effectiveness of medical care for cancer patients directly depends on the development and introduction of modern methods of nuclear medicine into medical practice.

Nuclear medicine techniques allow detection of functional abnormalities at early stages of disease, at the level of metabolic disturbances, thereby improving the likelihood of successful outcomes. However, the development of nuclear medicine imaging technologies in Russia is still far behind the world level. It is estimated that the demand for radiopharmaceuticals in Russia is satisfied by no more than 1–3%. In this regard, it seems relevant to develop new innovative radiopharmaceuticals for molecular imaging [1–3].

Glucose derivatives labeled with radioactive isotopes are promising radiopharmaceuticals for the early cancer detection [4]. This is due to the fact that malignant cells have an elevated level of glucose metabolism compared to normal cells. In addition, the number of specific glucose transporters has been increased in the cytoplasmic membranes of tumor cells, so the level of its entry into the cytoplasm is high. A variety of positron-emitting nuclides is currently utilized to detect tumors and metastases as well as to monitor treatment response. The most commonly used PET radiopharmaceutical is a derivative of glucose, namely 2-(18F) fluoro-2-deoxy-glucose (18F-FDG). Despite the high diagnostic value of 18F-FDG-PET, the widespread use of this imaging modality in Russia is limited because of its high cost and lack of PET centers. The estimated cost of building a PET center is about 1 billion rubles.

Currently, there are about 30 centers of positron emission tomography in Russia. However, along with a small number of PET centers, there are more than 200 SPECT centers (single-photon emission computed tomography). The most frequently used radionuclide for SPECT is technetium-99m. The main advantage of radiotracers based on technetium-99m-labeled glucose derivatives is the ability to image different tumor types using a gamma camera, thus significantly reducing the cost of the diagnostic procedure [5].

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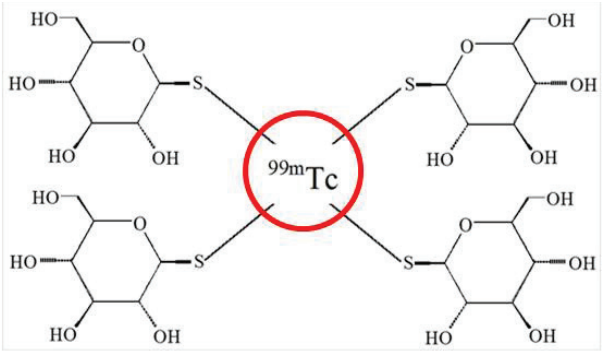

FIGURE 1. Structural formula of ^{99m}Tc-1-Thio-D-glucose

The development of new radiopharmaceuticals based on technetium-99m- labeled glucose derivative will increase the number of cancer-related studies and allow cancer at early stages to be detected.

MATERIALS AND METHODS

The purpose of the study was the development and preclinical study of ^{99m}Tc-1-Thio-D-glucose, a new rdiopharmaceutical based on technetium-99m-labeled derivative of glucose, for cancer visualization. The structural formula of ^{99m}Tc-1-Thio-D-glucose is shown in Fig. 1.

The ready-made ^{99m}Tc-1-Thio-D-glucose contains a solution of 1-thio-D-glucose in the presence of tin (II) chloride dihydrate ($SnCl2 \cdot 2H2O$). The radioactive label is technetium-99m from sodium pertechnetate solution (Na99mTcO4) from a sorption generator or sodium pertechnetate solution (Na99mTcO4) from an extraction generator. The composition of 99mTc-1-Thio-D-glucose is shown in Table 1.

Preclinical trials on the safety and efficacy of ^{99m}Tc-1-Thio-D-glucose were conducted in strict compliance with the guidelines for Preclinical Drug Development (A. Mironov). Toxicological studies of the new radiopharmaceutical included study of

– acute toxicity,
– cumulative effect,
– chronic toxicity,
– immune toxicity,
– mutagenicity,
– allergenicity,
– reproductive toxicity.

In addition, the pharmacokinetic parameters of ^{99m}Tc-1-Thio-D-glucose and its functional suitability were studied. The animals were kept in accordance with the rules adopted by the European Convention for the Protection of Animals (Strasbourg, 1986).

TABLE 1. The composition of ^{99m}Tc-1-Thio-D-glucose

Active substance	1 ml of the finished product contains
Technetium-99m as a complex with 1-thio-d-glucose	250–750 MBq
Excipients	1 ml of the finished product contains
^{99m}Tc-1-thio-D-glucose sodium salt hydrate	No more than 0.625 mg
Tin dichloride 2-aqueous	0.044–0.052 mg
Ascorbic acid	No more 0.125 mg
Sodium chloride	8.0–10.0 mg
Water for injections	Up to 1 ml

RESULTS

Study of Acute Toxicity of ^{99M}TC-1-Thio-D-glucose

The study of acute toxicity suggested a single administration of ^{99m}Tc-1-Thio-D-glucose with subsequent monitoring of animals for 14 days. The aim of the study was to evaluate the tolerable, toxic and lethal doses of intra-abdominal and intravenous injections of the radiopharmaceutical using the Litchfield and Wilcoxon methods, to determine the causes of death in animals and to study the effect of the radiopharmaceutical on functional and morphological parameters. The acute toxicity of ^{99m}Tc-1-Thio-D-glucose was studied in 80 rats (40 males and 40 females) and 80 mice (40 males and 40 females). No changes in appearance, motor activity and behavior of animals were revealed during a 14-day follow-up period. The animals were followed-up for the first 8 hours after administration of the radiopharmaceutical, then daily. The effect of the radiopharmaceutical on the behavior, feed and water intake, appearance, motor activity, and animal response to external stimuli was studied. No anomalies were found. The animals were weighted before the experiment, then again at the end. All of the animals gained weight. The toxic effect of the radiopharmaceutical was not revealed. A postmortem examination was conducted after the completion of the 14-day follow-up period. During the entire follow-up period, there was no animal death. During autopsy, no differences were found between the animals of the experimental and control groups. No macroscopic and microscopic pathological changes in the internal organs of animals were revealed. Thus, acute toxicity studies showed that intravenous and intraperitoneal injections of ^{99m}Tc-1-Thio-D-glucose were well-tolerated by rats and mice.

Study of the Cumulative Toxicity of ^{99M}TC-1-Thio-D-glucose

The cumulative toxicity of ^{99m}Tc-1-Thio-D-glucose was studied on 20 conventional outbred rats (10 males and 10 females), 5 animals per group. The radiopharmaceutical was administered to animals by intraperitoneal injection of 12.5 ml/kg (1/2 LD50) daily for 28 days. The control group of animals received the same volume of saline. During the study, the animals were weighed once a week and monitored for one week. During this period, no animal deaths were observed. In the experimental group of rats (males and females) receiving the radiopharmaceutical, no statistically significant changes in the body weight index were observed in comparison with the control animals. There were also no changes in the body weight gain of experimental animals during the same follow-up period. Postmortem examination showed that the organs of the thoracic and abdominal cavities had anatomically correct position and normal macrostructure. No macroscopic pathological changes were found. Thus, the results of the study indicate that ^{99m}Tc-1-Thio-D-glucose has no cumulative toxicity.

Study of Chronic Toxicity of ^{99m}Tc-1-Thio-D-glucose

The chronic toxicity of ^{99m}Tc-1-Thio-D-glucose was studied on 80 rats weighing 250–350 g (males and females) and 12 rabbits. The radiopharmaceutical was intravenously administered to rats at doses of 0.2 ml/kg, 5 ml/kg, and 10 ml/kg for 7 days. The animals were monitored for 30 days to assess their condition, weight and body temperature. The assessment of peripheral blood, bone marrow, liver, kidneys, nervous system and heart was also carried out. In addition, the morphological examination of the internal organs and their weight was performed. None of the animals died during the follow-up period. Macroscopic and microscopic assessment of the internal organs showed no abnormalities in the functional activity of their internal organs and systems compared to the control group of animals.

Thus, ^{99m}Tc-1-Thio-D-glucose did not exert a toxic effect on the organs and systems of the animals. The study of immune toxicity, mutagenicity, allergenicity and reproductive toxicity revealed no significant abnormalities in the examined animals and their progeny after the administration of this radiopharmaceutical.

Study of the Pharmacokinetics of ^{99m}Tc-1-Thio-D-glucose

The main goal of the study of the pharmacokinetics of ^{99m}Tc-1-Thio-D-glucose was to analyze the distribution and excretion of this radiopharmaceutical in the bodies of the laboratory animals. The main organs and tissues of laboratory animals, in which the radiopharmaceutical was most intensively accumulated after its intravenous administration, were determined.

FIGURE 2. The curve of ^{99m}Tc-1-Thio-D-glucose concentration in the blood plasma. The balance between plasma concentration of 99mTc-1-Thio-D-glucose and extravascular concentration occurs 10 min after the intravenous injection of the radiopharmaceutical

The ways of elimination of ^{99m}Tc-1-Thio-D-glucose from the animals' bodies were also identified. The study was conducted on 65 mature conventional outbred white male rats, males (weight: 250 ± 30 g, age: 10–12 weeks), and 9 Soviet chinchilla rabbits (weight: 2800–3100 g, age: 12–18 months).

In the first seconds after intravenous administration of ^{99m}Tc-1-Thio-D-glucose, it was quickly distributed in the blood plasma. The concentration of the radiopharmaceutical in the blood plasma began to decrease 1 minute after its injection. The radiopharmaceutical was actively subjected to glomerular filtration and was detected in the renal parenchyma and urine 1 min after the injection. The radiopharmaceutical did not penetrate through the intact blood-brain barrier, as evidenced by its insignificant concentration in the brain of animals, mainly due to the presence of blood in the membranes of the brain. The half-life of ^{99m}Tc-1-Thio-D-glucose from the blood plasma (T1/2) was 10 min (Fig. 2). It should be noted that the radiopharmaceutical was not detected in the blood plasma of laboratory animals 24 hours after the injection, that is, the radiopharmaceutical was completely eliminated from the blood plasma 24 hours after its injection.

The radiopharmaceutical was excreted by glomerular filtration. The mean concentrations of ^{99m}Tc-1-Thio-D-glucose in the main organs and tissues of laboratory animals in 30 s, 1, 3, 10 and 30 min, 1, 3, 10 and 24 hours after intravenous administration are shown in Table 2.

TABLE 2. The average concentration of 99mTc-1-Thio-D-glucose in organs and tissues of rats after single injection of this radiopharmaceutical (%/g)

Organs	30 s	1 min	3 min	10 min	30 min	1 h	3 h	10 h	24 h
Blood	13.856 ± 0.236	12.34 ± 0.517	9.378 ± 0.294	6.514 ± 0.367	1.876 ± 0.039	0.240 ± 0.014	0.0032 ± 0.004	0.000	0.000
Brain	0.0000	0.000	0.010 ± 0.014	0.012 ± 0.011	0.008 ± 0.011	0.000	0.0000	0.000	0.000
Heart	0.0800 ± 0.036	0.088 ± 0.045	0.098 ± 0.034	0.084 ± 0.035	0.116 ± 0.024	0.040	0.0000	0.000	0.000
Lungs	0.0260 ± 0.013	0.042 ± 0.022	0.036 ± 0.023	0.062 ± 0.018	0.038 ± 0.016	0.028 ± 0.011	0.0000	0.000	0.000
Liver	0.0016 ± 0.002	0.007 ± 0.003	0.092 ± 0.008	0.066 ± 0.005	0.028 ± 0.004	0.007 ± 0.007	0.0000	0.000	0.000
Spleen	0.0120 ± 0.016	0.018 ± 0.016	0.196 ± 0.047	0.135 ± 0.034	0.066 ± 0.013	0.000	0.0000	0.000	0.000
Small intestine	0.0000	0.000	0.000	0.000	0.000	0.000	0.0000	0.000	0.000
Colon	0.0000	0.000	0.000	0.000	0.000	0.000	0.0000	0.000	0.000
Kidneys	0.388 ± 0.018	1.894 ± 0.069	4.540 ± 0.378	4.340 ± 0.247	3.364 ± 0.021	0.408 ± 0.029	0.0040 ± 0.009	0.000	0.000
Urine	0.0000	3.082 ± 0.193	6.146 ± 0.322	18.338 ± 1.019	28.500 ± 0.757	39.004 ± 1.595	30.990 ± 3.761	21.996 ± 0.304	2.596 ± 0.036
Muscle	0.0000	0.000	0.104 ± 0.015	0.096 ± 0.017	0.064 ± 0.017	0.008 ± 0.010	0.0000	0.000	0.000

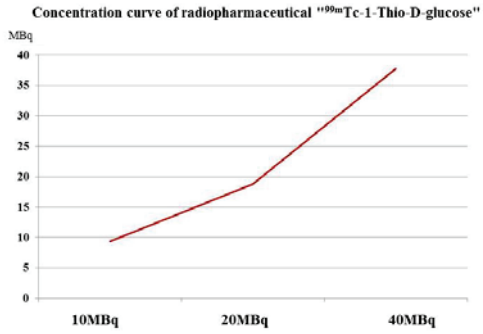

FIGURE 3. The curve of the concentration of ^{99m}Tc-1-Thio-D-glucose in the blood plasma of rats at different dose levels (10, 20 and 40MBq)

The data obtained confirmed the linearity of pharmacokinetics of 99mTc-1-Thio-D-glucose. It was found that the concentration of the radiopharmaceutical in the blood plasma of laboratory animals proportionally increased with increasing doses. The pharmacokinetic curve of 99mTc-1-Thio-D-glucose is shown in Fig. 3.

The distribution and accumulation of ^{99m}Tc-1-Thio-D-glucose, which was injected for 5 days, corresponded to the distribution of the radiopharmaceutical after its single injection, thus indicating that ^{99m}Tc-1-Thio-D-glucose did not have cumulative effect and did not accumulate persistently in the main organs and tissues of the animals.

Study of Potential Utility of ^{99m}Tc-1-Thio-D-glucose

The potential utility of ^{99m}Tc-1-thio-D-glucose was studied in vitro and in vivo models. In vitro studies, cell cultures from normal (Chinese hamster ovary (CHO) cells) and cancer tissues (MCF 7–human breast adenocarcinoma cell line) were used. 50 MBq of ^{99m}Tc-1-thio-D-glucose was added to vials with 1,000,000 cells/ml, 5 vials for each cell culture. The vials with cells were then incubated for 40 minutes at room temperature with periodic gentle shaking to avoid cell adhesion. After completion of incubation, the vials were centrifuged at 300 rpm for 5 min. After sedimentation, the supernatant was removed and the radioactivity measurement of the sediment and supernatant was made. Thereafter, cells were washed three times by adding 1 ml of buffer to each vial. PBS + 1% BCA were used as a buffer.

After the final cell washing, the ^{99m}Tc-labeled 1-thio-D-glucose uptake in normal and cancer tissue cells was measured using E.CAM180 gamma camera (Siemens, Germany). The study results demonstrated that the ^{99m}Tc-labeled 1-thio-D-glucos uptake in cancer cells (MCF 7) was 2 times higher than that in normal tissue cells (CHO). The mean radioactivity levels in cancer and normal tissue cells after the final washing were 7.21 ± 0.4 and 13.7 ± 0.6, respectively. Planar scintigraphic images of plates with culture vials showed more intense radiotracer uptake in cancer tissue cells than in normal cells (Fig. 4).

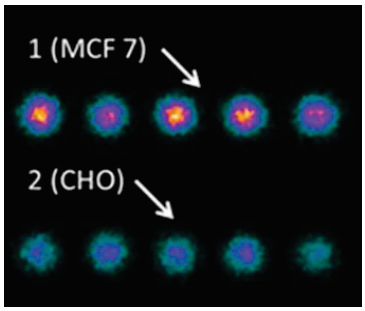

FIGURE 4. Scintigraphic imaging of the ^{99m}Tc-1-Thio-D-glucose uptake: *1*—cancer tissue cells, *2*—normal tissue cells

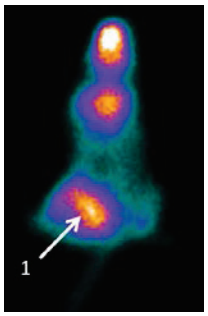

FIGURE 5. Scintigraphic image of a tumor bearing mouse at 15 min after intravenous injection of ^{99m}Tc-labeled 1-thio-D-glucose. High ^{99m}Tc-1-thio-D-glucose uptake in the tumor of the right femur (*1*)

In vivo studies, the potential utility of 99mTc-labeled 1-thio-D-glucose was studied using the intramuscularly implanted solid Lewis lung carcinoma model in C57B1/6J mice. A total of 50 male and female mice, weighing 28 ± 5g and 8–10 weeks of age, were used. The median tumor size in mice was 1.67 ± 0.12 cm^3. The mice were anesthetized by inhalation of ethyl ether. The radiopharmaceutical at a dose of 20 MBq was intravenously injected to C57BL/6J mice bearing Lewis lung carcinoma and to the control group mice. Whole-body gamma scintigraphy using the e.cam® Siemens dual-head 180° gamma camera was performed 40 min after intravenous injection of the radiopharmaceutical. The mice were placed with their ventral surface to the gamma camera detector so that the imaging field of view could encompass the whole body of the animal. Each image was acquired for 5 min. About 500,000 counts were recorded in each image with matrix size of 256×256 pixels. The high-resolution, low-energy collimators for 140 keV energy were used. Statistical analysis was performed using E.Soft software (SIEMENS, Germany).

Scintigraphic images demonstrated extremely high ^{99m}Tc-1-Thio-D-glucose uptake in tumor tissue of all mice bearing Lewis lung carcinoma (Fig. 5). In the control group animals, the radiopharmaceutical accumulated more intensively in the kidneys and bladder. The tumor-to-background ratio was 4.15 ± 0.35. As a background, a contralateral region of a healthy limb was used.

CONCLUSION

In the preclinical studies, ^{99m}Tc-1-thio-D-glucose demonstrated no toxic effect on the main organs and systems of laboratory animals. Based on the results of toxicological studies, ^{99m}Tc-1-Thio-D-glucose was classified as a class of low-toxic substances.

It should be noted that ^{99m}Tc-1-Thio-D-glucose has optimal physical/chemical and pharmacokinetic parameters for the diagnostic imaging. This radiopharmaceutical does not penetrate through the intact blood-brain barrier and does not accumulate persistently in the main organs and tissues. ^{99m}Tc-labeled 1-thio-D-glucose is excreted from the kidneys by glomerular filtration.

In vitro study of potential utility of ^{99m}Tc-1-Thio-D-glucose for tumor visualization showed more intense radiotracer uptake in cancer tissue cells than in normal cells. In vivo studies indicated that the ^{99m}Tc-1-Thio-D-glucose uptake in tumor tissues was significantly higher than that in intact tissues.

Thus, ^{99m}Tc-1-Thio-D-glucose, a new tumor-seeking agent based on technetium-99m-labeled glucose derivative, is a promising radiopharmaceutical for cancer visualization by SPECT. It is characterized by low cost of production and ease of use in the standard nuclear medicine laboratory.

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