Development new radiopharmaceutical based on 5-thio-d-glucose labeled technetium-99m

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Abstract. The article considers the obtaining and possibility of using 5-thio-D-glucose labeled technetium-99m for the diagnosis of malignant tumors by single photon emission computed tomography. The analysis of the level of international developments of radiopharmaceuticals based on derivatives of glucose has been carried out. Also the article provides information on of using experimental batches of lyophilisate on the basis of 5-thio-D-glucose for preliminary biomedical testing on the mice.

1. Introduction

Timely performed diagnosis and detection of malignant tumors in the early stages of development remains one of the most important problems of modern medicine. Over the past 10 years, according to the Ministry of Health and Social Development, the death rate from cancer has increased to a level of 13.87% and has become the second leading cause of mortality in Russia. Annually in our country about 480 thousand cases of cancer tumors are revealed. Unfortunately, about 60% of registered patients are detected in the late stages of disease, and this greatly reduces the possibility of treatment. Thus, in 2013 the death rate from cancer in Russia amounted to 292 thousand people (according to the Federal State Statistics Service on 05.22.2014). Therefore, the efficiency of medical care to patients with cancer depends on the level of development and introduction of modern nuclear medicine techniques into medical practice. The uniqueness of nuclear medicine techniques is that they allow to diagnose functional abnormalities of vital activity of organs at the earliest stages of the disease when a person does not feel any symptoms. This makes it easier to detect and treat a wide variety of diseases, which significantly increases the likelihood of cure.

Glucose derivatives, labeled with radioactive isotopes, are promising radiopharmaceutical (RPC) for early diagnosis of malignant neoplasms. This is due to the fact that in tumor cells there is an increased level of glucose metabolism as compared to normal cells [1]. In tumor cells the number of glucose transporters is increased, so glucose administration to the cancer cells also increases [11,12].

There are different glucose derivatives labeled with radionuclides ^{18}F, ^{11}C and others. Currently in Russia in Positron Emission Tomography (PET) a radiopharmaceutical 2-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is used. This preparation comprises a positron-emitting radionuclide fluorine-18, for the diagnosis of tumors, metastases and evaluating of the anticancer therapy efficiency. Despite the high diagnostic informative value of PET with ^{18}F-FDG, the widespread use of this method is limited because of its high cost and the lack of PET centers in most regions of Russia.

The main advantage of RPC based on glucose derivatives labeled with technetium-99m, is that visualization of tumors and their use can be made by single-photon emission computed tomography (SPECT), which significantly reduces the cost of the diagnostic procedure. Now in Russia there are over 200 SPECT centers, and the most frequently used radionuclide for SPECT studies is technetium-99m. Therefore, the aim of this work is to study the possibility of obtaining technetium-99m-labeled glucose derivatives, such as 5-thio-D-glucose for the subsequent creation of a new radiopharmaceutical on its basis.

2. Methods of synthesis of radiopharmaceutical "2-fluoro, ^{18}F-2-deoxy-D-glucose"

For the first time [^{18}F] FDG was synthesized more than 35 years ago at the Brookhaven National Laboratory (USA) using the method of electrophilic fluorination [13]. But, unfortunately, it is not widely used due to the complexity of the synthesis. In 1986, a group of German scientists [14] have developed a method of obtaining a nucleophilic [^{18}F] FDG. This method proved to be more simple to perform and has a higher radiochemical yield.

Preparation of 2-fluoro, ^{18}F - 2-deoxy-D-glucose in using nucleophilic radiofluorination [15] is carried out starting from 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose (TATM) which is synthesized by Hamacher [16] of 1,3,4,6-tetra-O-acetyl-beta-D-mannopyranose and its - from D-mannose [17]. The complete synthetic scheme is shown in Figure 1.

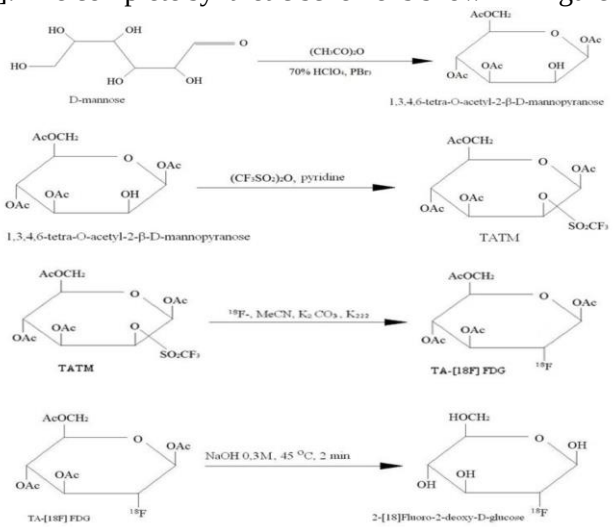

Figure 1. Scheme nucleophilic synthesis of 2-fluoro [^{18}F]-2-deoxy-D-glucose.

Before the introduction of fluoride anion ^{18}F into TATM, it is pre-separated from the target water using microcolumn filled with resin QMA Accel Waters or other anion-exchange resin with similar properties. Desorption of fluoride from the column is carried out with an eluent consisting of kryptofix, potassium carbonate, deionized water and acetonitrile. The result is a complex of fluoride-ion with a catalyst in a mixture of acetonitrile/water, which is distilled before the fluorination as an azeotropic mixture under a stream of nitrogen.

The substitution reaction of a triflate group in TATM to ion fluorine -18 is conducted in acetonitrile solution at 80°C. Its radioactive product is 1,3,4,6-tetra-O-acetyl-2-^{18}F-β-D-glucopyranose. Hydrolysis of the reaction mass is carried out at alkaline catalysis (solution 0.3 M NaOH) at 45°C for 2 min. The result is 2-fluoro-2-deoxy-D-glucose, labeled with radionuclide^{18}F.

After neutralizing of the mixture with 0.5 M HCl solution, it is heated in the reaction vessel to 120°C and under a nitrogen stream the traces of acetonitrile are removed during 3-4 min. Reaction mixture diluted with water is transferred to purification column filled with a cation-exchange resin Supelclean LC-SCX, Supelco and neutral aluminum oxide. After purification, the isotonicity of a resulting preparation is achieved by mixing it with an aqueous solution of sodium chloride, in such a

concentration that NaCl content of the finished RPC is 8-10 mg/ml. Then sterilization is performed by passing it through a sterilizing Millipore filter with a pore diameter of 220 nm. All these chemical operations can be performed by a laboratory robot RB-86 Anatech («Scanditronix», Sweden).

A synthesis scheme, shown in Fig. 1, is used in radiochemistry laboratory of the Institute of Human Brain of RAS (St. Petersburg) [18].

This method of synthesis of ^{18}F-fluorodeoxyglucose is quite complex and requires the use of special equipment, such as a cyclotron, a radiochemical module or a laboratory robot along with the PET camera costing $ 5 million. Furthermore, the reaction with the given precursor cannot be implemented with other radionuclide, such as iodine-123 or technetium-99m, convenient for conducting research using wide-spread gamma-cameras [2].

3. Methods for obtaining derivatives of glucose labeled with technetium-99m

In [3] it is shown that for tumor lyophilisate, using a gamma camera, the complexes of ^{99m}Tc with a variety of glucose derivatives can be used. Here, for the labeling by isotope ^{99m}Tc, the authors tested glucose derivatives, which contain nitrogen atoms in their composition. The preference was given to D-glucosamine, its salts and hydrates.

^{99m}Tc-labeled complexes of glucose derivatives had radiochemical purity (RCP) of 98% or more. Approximately the same parameters of RCP were achieved in study [4], when mixing 5-thio-D-glucose with sodium pertechnetate, ^{99m}Tc (1.85-3.7 GBq) in the presence of 0.01 mg of tin chloride (II) and subsequent 30-minute incubation of the mixture at room temperature. Tests of the obtained preparation in rabbits showed a level of binding with proteins of 32%, which is favorable for the detection of tumors.

Also, good results were obtained in [5], which describes the process of radiolabeling with ^{99m}Tc and preliminary biological tests of complex of dietelentriaminepentaacetic acid (DTPA) with deoxyglucose (DG) were conducted.

In the first step of this process, the reduction of ^{99m}Tc with tin chloride (II) was conducted. Thereafter , reduced technetium-99m was added to the complex of DTPA-DG, and incubation of the mixture was carried out at room temperature for 30 min. Conducted biological tests have shown that the absorption coefficient of brain tumor of the complex ^{99m}Tc-DTPA-DG in relation to the muscles was higher than that of ^{18}F-FDG. At the same time in any other organs no significant accumulation was observed. And it has been revealed that blood is rapidly cleaned through kidneys [6].

Good quality of tumor images made by the method of SPECT is also observed when using labeled glucose analog ^{99m}Tc-glukarate [7]. In another work [8] the authors studied the differences in the biological behavior of ^{99m}Tc-labeled 1-thio-beta-D-glucose 2,3,4,6-tetra acetyl analog (Tc-TG) and ^{18}F-FDG. Binding of both indicators was performed in vitro on viable tumor cells and bacteria. Both indicators were then injected into the thigh muscle of mice, which were infected with Staphylococcus aureus. Thus, it was shown that both indicators effectively bind tumor cells and provide a high accumulation ratio in infected muscle tissues. Both tracers are rapidly removed from the blood system through the kidneys, mainly accumulating in the bladder, followed by their removal from the body.

In the animal tumors one could observe a high level of accumulation during administration of technetium-99m-labeled glucose analog ^{99m}Tc-ethylene ditsestin deoxyglucose (ECDG), which was obtained from the reaction of ethylene ditsestin with glucosamine in the presence of a carbodiimide coupling agent. [9]

4. Experimental results and discussion

The most of diagnostic drugs based on technetium-99m in clinical conditions is received by mixing of eluate from the generator ^{99m}Tc with standard sets of reagents, which are intended for receiving the set radiopharmaceutical. As such standard sets to ^{99}Mo/^{99m}Tc the ^{99m}Tc-generator the lyophilisate representing the lyophilised mix of components of a dosage form of drug are used. The reducer - a dihydrate of tin chloride (II) ($SnCl_2 \cdot 2H_2O$) which in standard conditions quickly is oxidized is a part of lyophilisate, but at a freeze-drying in vacuum keeps the properties within a year that is the regulated

period of validity of lyophilisate.. For development of new radiopharmaceutical based on glucose derivatives of 5-tio-D-glucose was taken as a basis. The structural formula of 5-tio-D-glucose has an appearance.

At an initial stage of researches solubility of the chosen substance in various environments of an organic or inorganic origin, for the purpose of selection of solvents and system for a chromatography was checked. As solvents tested the following solutions: normal saline solution – 0.9% NaCl solution, H_2O (a dist.), acetone, ammonia, the dehydrated alcohol, 75% alcohol solution.

For check of solubility of substance of 5-tio-D-glucose add hinge plates on 5 mg in separate bottles, and then entered the prepared solvents of 1 ml into each of bottles. Solubility of substance was defined visually with use of the specialized installation representing the light stand with the optical device. Results are provided in table 1.

Table 1. Solubility of 5-tio-D-glucose in different solvents.

Solvent	Result of process of dissolution	pH of mixes
0.9% NaCl solution	Dissolves without being heated, the solution becoming clear	5.0
H_2O (dist.)	Dissolves without being heated, the solution becoming clear	5.0
Acetone	Does not dissolve, even when heated up	6.0
The dehydrated C_2H_5OH	Slightly soluble	6.5
C_2H_5OH - 75% solution	Dissolves without being heated, the solution becoming clear	6.5
0.5 M NaOH solution	Dissolves without being heated, the solution becoming clear	12.0
0.5 M HCl solution	Dissolves without being heated, the solution becoming clear	1.5
Ammonia	Dissolves without being heated, the solution becoming clear	1.5

By results of the conducted researches the conclusion was drawn that substance 5-tio-D-glucose in dehydrated alcohol is poorly soluble, but is well soluble in 75% spirit. In alkaline condition, substance though is completely soluble, but over time collapses. Full dissolution of 5-tio-D-glucose without formation of a colloid is observed in normal saline solution, water and muriatic solution.

Now rather easy way of receiving 5-tio-D-glucose with technetium-99m is known [4], according to which the drug of ^{99m}Tc-5-tio-D-glucose prepare by mixing 10 mg of 5-tio-D-glucose from 0.074 mg of tin chloride (II) of a dihydrate with the subsequent introduction to the received mix of 2-4 ml of solution of a pertechnetate of ^{99m}Tc with activity of 50-100 mCi (1.85-3.7 GBq) and an incubation within 30 min. Thus efficiency of branding makes 98.5 ± 0.8 % and remains stable 24 hours. Authors do not consider possibility of preparation of lyophilisate therefore original stock of reagents for receiving drug cannot be stored more than 1-2 hours, owing to oxidation of tin (II) to the 4th valence state.

Approximately same technique of preparation of the marked ^{99m}Tc 5-tio-D-glucose it is offered in [19]. Here in the bottle containing 5-tio-D-glucose mix (5 mg, 0.51 mmol) and $SnCl_2 \cdot 2H_2O$ (0.080 mg), add eluate $^{99m}TcO^{4-}$ at the rate of 20 mCi/ml. After mix with in 10 min.

As experimental check showed, at preparation from these structures of lyophilisate, the exit of a marked main product in filtrates of 200 nanometers did not exceed the 40th percent. Most part of activity ^{99m}Tc, it was adsorbed on a colloid. Thus on chromatograms of the received radiopharmaceutical in Acetone it was found in addition to peak ^{99m}Tc (VII) two peaks of initial substance of 5-tio-D-glucose that gave the grounds to assume that at process of freeze-drying there is its partial decomposition. Technetium eluate for research was obtained from technetium generators "^{99m}Tc-GT-TOM" and "GT-TOM-II».

For 5-tio-D-glucose substance stabilization into composition of reaction mixture it was solved to enter as the stabilizing additive ascorbic acid in number of 0.5 mg. For its preparation, the hinge plate of ascorbic acid weighing 100 mg was dissolved in 10 ml of water (concentration of 10 mg/ml). Besides, influence of pH of environment at a size of a radiochemical exit of a main product was studied. Hydrogen ionization value of reagent before freeze-drying of mix established by introduction to its structure salt 0.05 M of solution of hydrochloric acid in quantity to 0.36 mg/ml. 5% $NaHCO_3$ solution used for increase of pH to values 5 and more.

As showed researches, optimum value rn to mix at which the maximum radiochemical exit of a main product is reached (to 98%), is in limits 3.5 – 4.0. The increase in pH to 5.0 by introduction to mix of a hydrocarbonate of $NaHCO_3$, led to noticeable decrease in an exit of a marked complex almost by 18% though radiochemical purity thus remained on rather high level (96%).

Radiochemical purity (RCP) of the received product was estimated by removal chromatogram in two mobile phases: in acetone (R_f = 0.9 $^{99m}TcO_4^-$) and in the mix C_2H_5OH : 25% of NH_4OH: H_2O ÷ 2 : 5 : 5 (R_f = 0.0 radiocolloids) with use of plates of PTLC silicagel-AF-A-UV (Sorbfil), the mm size 20×100. Putting drug on plates, in number of 5 μl, was carried out having receded from one of edges on 20 mm (the line of start). After drying of a spot on air the strip was placed in the camera for a chromatography for 10-40 min, depending on the used mix. Visualization and studying of distribution of activity ^{99m}Tc on length chromatogram carried out by means of the «Gammaskan-01A» installation. The radiochemical purity of 5-thio-D-glucose was calculated by subtracting from 100% the percents of free pertechnetate and radiocolloids.

Due to introduction to composition of reaction mixture of the ascorbic acid and strengthening of a reducer it was succeeded to increase stability of substance and as a result, on chromatogram in acetone the second peak of a product of decomposition was not found.

Finally, the following technique of preparation of drug was developed. A hinge plate of 5-tio-D-glucose weighing 15 mg entered in a bottle with a capacity of 10 ml and parted it in 1 ml of water for injections. Then consistently added to a bottle 20-25 μl freshly cooked Sn (II) solution (concentration of 7 mg/ml on $SnCl_2 \cdot 2H_2O$), 45-50 μl aqueous solution of ascorbic acid with concentration of 10 mg/ml and 200 μl, 0.05M of HCl. The received solution is passed via the sterilizing filter (pore size of 0.22 microns) in a sterile bottle with a capacity of 10 ml. After a bottle without prefreezing of mix placed in the refrigerator of a lyophilizer and carried out process of freeze-drying in the automatic mode within 24 hours.

The 4 ml of eluate ^{99m}Tc with activity of 1.5-2 GBq (40-50 mCi) entered into a bottle with reagent for the subsequent preparation of drug. The drug was ready to use incubation of mix at the room temperature in 30 min.

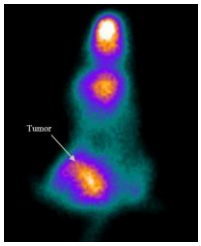

Figure 2. Scintigram of a mouse obtained in 30 min after administration of radiopharmaceutical based on 5-tio-D-glucose marked by technetium-99m.

Also studying of stability of the received radiopharmaceutical in time on an indicator radiochemical purity was carried out. Definition of RCP was carried out each 30 min within 7 hours by according to the described technique since the moment of preparation of RP. The received results showed that within 4 hours radiochemical purity to be in limits of 95-98 %, but in 4.5 hours falloff of radiochemical purity begins and by the end of the 6th hour it falls to 89 %. From what the conclusion was drawn that period of validity of a drug on an indicator of radiochemical purity makes 4 hours.

Preliminary biomedical testing of the received radiopharmaceutical ^{99m}Tc-5-tio-D-glucose, which are carried out at the Tomsk Cancer Research Institute on the mice of the C57BL/6 line with Lewis's carcinoma (LLC) graft in hip area showed functional suitability of a drug. Level of accumulation of drug in a malignant tumor made 6.5 % of the general entered activity that is enough for its reliable visualization. Scintigram of a mouse body obtained in 30 min after administration of radiopharmaceutical based on 5-tio-D-glucose labeled by technetium-99m, presented on figure 2.

5. Conclusion

As a result of experiments it has been established that:

1. Development of the composition and methods of preparation of the radiopharmaceutical formulation "^{99m}Tc-5-thio-D-glucose" in the form of lyophilisate.
2. The radiochemical purity of technetium-99m-labeled preparation, produced on the basis of lyophilizates of 5-thio-D-glucose, is in the range of 95-98 %.
3. Preparation without noticeable decrease in its radiochemical purity remains stable for 4 hours.
4. Preliminary biomedical testing the resulting radiopharmaceutical conducted at the Tomsk Cancer Research Institute in the mice C57BL line / 6 mice with tumor hip. It is confirmed its functional suitability.

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