ANOTATION

Dissertation for the degree of Doctor of Philosophy (Ph.D.) in the specialty "6D060600 - Chemistry"

GURIN ANDREY NIKOLAEVICH

Development of a radiopharmaceutical based on Lu-177 labelled elagolix for therapy and diagnosis

General characteristics of research work

The thesis «Development of a radiopharmaceutical based on Lu-177 labelled elagolix for therapy and diagnosis» is devoted to the development of a procedure for producing a radiopharmaceutical based on antagonist of gonadotropin-releasing hormone (GnRH) for the diagnosis and treatment of triple-negative breast cancer.

The relevance of the study

Breast cancer (BC) is a malignant neoplasm of the glandular tissue of the breast. Despite the decrease in mortality due to the introduction of preventive examinations and effective systemic hormonal chemotherapy, about 1 million new cases are registered annually in the world.

In women in many countries of the world, including Kazakhstan, breast cancer (BC) ranks 1st among other types of malignant neoplasms. On average, about 4 000 breast cancer patients are diagnosed annually in the Republic of Kazakhstan, and more than 1 380 women among them die. In particular, 4 142 new cases of breast cancer were registered in 2014, which amounted to 22.4 per 100 000 of the population. The lethality for 1 year is 8.2%, and the 5-year survival rate is 55.8%. The specific weight of BC I-II stages in 2013 was 77.1%, and BC IV stage was 4.9%**.**

Triple negative breast cancer (TNBC) accounts for 8-20% of breast tumors. A special feature is that triple negative breast cancer occurs in women under the age of fifty years before menopause and even during the first pregnancy in the early period, and then after childbirth, it may be accompanied by short-term breastfeeding. Also occurs early in menarche and with high body mass index. Cells of this type are characterized by a lack of expression of receptors for estrogen, progesterone and HER-2, which makes it difficult to choose a therapeutic vector that is aggressive, the maximum risk of recurrence within three years after surgery, metastasis, and a decrease in life expectancy.

The thrice negative phenotype includes a breast tumor subtype that is clinically negative for expression of estrogen and progesterone (ER and PR) receptors and is negative for the human epidermal factor (HER2) receptor protein, with unique prognostic and therapeutic indices. Unlike other subtypes, the target agents specifically targeted for thrice-negative breast cancer are

not yet available, which increases the need and interest in advancing new therapeutic strategies beyond the chemotherapy for this subgroup of high-risk patients.

Modern world treatment protocols provide for surgical intervention in order to remove the primary tumor and part of the lymph nodes into which malignant cells are likely to have spread. A combination of several treatments is also performed, such as hormone therapy, chemotherapy and radiation therapy. After surgery to remove the removal of breast cancer, there are no less important stages, such as high-precision radiation therapy with chemotherapy. Unfortunately, side effects limit the effectiveness of chemo-radio-therapy. The use of selective drugs makes it possible for effective treatment, which includes the determination of biochemical processes, as a result of which there is a differentiation of tumor tissue from healthy tissue.

The thrice negative breast cancer expresses the receptors for GnRH in more than 50% of cases. Among several analogues (agonists and antagonists) of GnRH, that have been studied for therapy, the most interesting is the non-peptid antagonist-elagolix. Elagolix is the first of a new class GnRH inhibitors that were designated as the second generation due to their non-peptidic nature and peroral bioavailability. Due to the relatively short half-life of elagolix, the action of GnRH is not completely blocked during a day period. For this reason, the levels of gonadotropin and sex hormones are only partially suppressed, and the degree of suppression can be dose-dependent adjusted when required. Moreover, if administration of elagolix is stopped, its effects are quickly reversible. Moreover, the frequency and severity of menopausal side effects, such as hot flashes, also decrease compared to the first-generation GnRH inhibitors.

The purpose of the thesis is radiolabeling of DOTAELA with ^{177}Lu with the following development a potential radiopharmaceutical for diagnosis and treatment of triple-negative hormone-sensitive breast cancer.

The tasks of the thesis

Production of lutetium-177 with high and proper specific activity by neutron activation for further using in radiolabeling;

Radiolabeling DOTAELA with produced lutetium-177 and determination the optimal synthesis of the DATOELA-^{177}Lu complex condition with high radiochemical purity

Selection and verification of quality control methods and preparation of draft specifications for the production of pilot batches.

The object of the thesis is radiometallic chelate for the diagnosis and treatment oftriple-negative hormone-sensitive breast cancer based on the elagolix - antagonist of gonadotropin-releasing hormone.

The subject of the research is the interaction of radioisotopes with chelating agents, determining the technological parameters of the synthesis at the product yield, quality control of the drug being developed.

The scientific novelty of the thesis

1. A paper chromatography system was selected to evaluate the yield of ^{177}Lu-DOTAELA. In the course of the work, such factors as chromatography paper, mobile phase, pH, composition and ratio of the components of the mobile phase were studied. This system will be further used in assessing the radiochemical purity of the ^{177}Lu-DOTAELA complex.
2. Optimal technological parameters of ^{177}Lu-DOTAELA synthesis were determined
3. The composition of the reaction mixture and the block scheme for producing a radiopharmaceutical based on DOTAELA have been developed. The finished product has a radiochemical purity of greater than 95%, which proves its suitability for medical and biological tests.
4. Methods of analytical quality control of the radiopharmaceutical "^{177}Lu-DOTAELA" have been developed. Approbation methods of qualitative and quantitative determination of the main components in the composition. A draft specification for a new radiopharmaceutical has been created.

The methodological framework of the research

Investigations were carried out with the help of generally accepted scientific and experimental methods. The experiments to determine the effect of temperature, time and composition on the technological output of the synthesis reaction, as well as testing of the optimal method for purifying of the synthesized batches, was performed in a sealed "hot" cell. To determine the technological output at the synthesis stage, the gamma spectrometry methods (gamma spectrometer EGPC 30-185-R), thin-layer chromatography was used to determine the radiochemical purity, the effect of the solvents composition and their residual quantity in the product, the method of gas chromatography (Agilent 7890A gas chromatograph GC), as well as high-performance liquid chromatography was used for determination of chemical purity (Agilent 1260). Apyrogenity was determined with the help of the LAL-test, and the sterility was determined by direct seeding on nutrient media, followed by incubation of the samples for 14 days at 25 °C and 37 °C

The scientific and practical significance of the study

The radiopharmaceutical material will find application in nuclear medicine for the diagnosis and treatment of TNBC. The results of this work will stimulate the development of highly informative and minimally invasive methods of nuclear medicine and contribute to the creation of new domestic RP for the diagnosis and treatment of various diseases.

The theoretical significance of the results

In the process of completing the thesis, a procedure was developed for the preparation of the dosage form of the radiopharmaceutical «^{177}Lu-DOTAELA», methods for monitoring its quality of the main components, and a draft Specification was proposed for the production of pilot batches of the radiopharmaceutical to conduct its subsequent preclinical studies at the University of Oslo.

The validity and reliability of the results

All results are reliable and reasonable since all measurements were carried out on calibrated instruments using standard methods using gamma spectrometry, atomic emission spectrometry, and paper chromatography. All conclusions are compared with the facts of the experiment.

Part of the study was carried out at the University of Oslo (Norway) under the supervision of Professor Patrick Riss.

The validity and reliability of the results are provided by metrological processing of the results of experimental data.

The main provisions submitted to the defense of the thesis

1. Obtaining the source of ^{177}Lu with the desired activity and purity, for elagolix labeling, produced by the neutron activation reaction through ^{176}Lu (n, γ)^{177}Lu at the WWR-K reactor with specific activity 819 GBq / mg Lu-177 with a thermal neutron flux of $1.2 \cdot 10^{14}$ $cm^{-2} \cdot s^{-1}$.

2. Optimal parameters for the synthesis177Lu radiolabeled DOTAELA at pH-4.5 with radiochemical yield 50.1 %, temperature 90-100° C with radiochemical yield 95.3%, complexation time 40 minutes with radiochemical yield 50.1%.

3. A block diagram of the 177Lu-DOTAELA complex preparation, showing target preparation, synthesis conditions and packing leading to RCP ≥ 95% and a draft specification for the development of its pilot batches.

Approbation of work

The results of the thesis were reported and discussed at international symposia and scientific conferences such as:

- 11th International Conference «Nuclear and Radiation Physics», September 12-15, 2017 in Almaty, Kazakhstan;

- International scientific conference of students and young scientists «FarabiӘlemi», Almaty, Kazakhstan, April 9-12, 2018;

- 19th reporting scientific and technical conference of the Institute of Nuclear Physics, December 18-19, 2018 in Almaty, Kazakhstan;

- III international scientific-practical conference «Actual problems of the development, production and use of radiopharmaceuticals - RADIOFARMA-2019», Moscow, June 18 - 21, 2019;

- I international conference «Nuclear and Radiation Technologies in Medicine, Industry, and Agriculture» June 24-27, 2019 in Almaty, Kazakhstan;

- The 20th reporting scientific and technical conference of the Institute of Nuclear Physics, December 18-19, 2019 in Almaty, Kazakhstan.

Publications

Based on the results of the thesis 10 scientific papers were published, including:

Gurin A.N., Patrick J Riss, Chakrova E.T., Uralbekov B.M. Development of quality control of the DO3A-NBI-56418 labeled by ^{177}Lu for theranostic goals of triple negative cancer with using paper chromatography // 11th International Conference "Nuclear and Radiation Physics", Almaty, 2017

Gurin A.N., Soloninkina S.G., Riss P., Uralbekov B.M., Matveyeva I.V., Chakrova E.T. Selection of mobile phase systems for chromatographic research of ^{177}Lu-DOTAELA. // Chemical journal of Kazakhstan. - 2018.- Vol. 2. - P. 151-157.

Gurin A.N., Soloninkina S.G. 3rd International Scientific Conference of Students and Young Scientists «Farabi Alemi», Almaty, Kazakhstan, April 9-12, 2018. «On the issue of the influence of pH synthesis on the radiochemical purity of the labeled compound DOTAELA - ^{177}Lu», 6 section, p. 245.

Gurin A.N., Chakrova E.T., Riss P. Purification of DOTAELA labeled with^{177}Lu // Proceedings of the III international scientific-practical conference «Actual problems of the development, production and use of radiopharmaceuticals - RADIOFARMA-2019» / Ed. G.E. Kodina and A.Ya. Maruk - M.: FSBI SSC FMBC them. A.I. Burnazyan FMBA of Russia, 2019.- P. 61.

Gurin A.N., Chakrova E.T., Riss P. Study of the stability of the^{177}Lu-DOTAELA // Abstracts of the 1st international conference «Nuclear and Radiation Technologies in Medicine, Agriculture Industry» - Almaty. - 2019. P. 219.

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. Quality control test for ^{177}Lu-DOTAELA. International Journal of Biology and Chemistry, [S.l.], v. 12, n. 2, p. 112-115, 2019. ISSN 2409-370X.

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. et al. Study of the purification of ^{177}Lu-DOTAELA complex // Pharmaceutical Chemistry Journal. 2020. Vol. 54. No. 1, P. 64-68 (Q4, IF – 0.51).

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. Development of a ^{177}Lu radionuclide-labeled bioconjugate, a gonadotropin releasing hormone antagonist // Medical physics. 2020. Vol. 85. No. 2, P. 17-23.

Gurin A.N., Riss P., Chakrova Ye.T., Matveyeva I. V. Optimization of Reaction Parameters for the Synthesis of ^{177}Lu DOTAELA // Revista de Chimie. 2020.Vol. 71. No. 8, P. 55-62.

Gurin A.N., Chakrova Ye.T., Matveyeva I. V. Patent №5186 for utility model «Method for radiochemical labeling of an organic compound with the lutetium-177 isotope», application No2020/0213.2 dated 27.02.2020 of RSE «National institute of intellectual property» (Republic of Kazakhstan).

The personal contribution of the author

Direct participation in research and the general formulation of tasks, in the analysis and statistical processing of the results; writing articles and reports, testing and introducing research results into the development of a new domestic radiopharmaceutical.

Relation of the thesis with research and government programs

This dissertation work was performed and supported within the framework of the grant funding project of MES of Republic of Kazakhstan AP 05134384 «Determination of the optimal technological parameters for preparation of a new radiopharmaceutical for diagnosis and therapy of thrice-negative breast cancer (TNBC) with an elagolix-^{177}Lu of antagonistic mechanism of action» (2018-2020 yy.).

Volume and structure of the thesis

The thesis consists of an introduction, three sections, a conclusion, and a list of references. The work is presented on 100 pages (with appendix 103), contains 38 figures, 16 tables, and 137 bibliographical references.