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Conformity with the principles of ethics. The patients signed an informed consent to participate in the study. The study was approved by the Bioethical Committee of the Cancer Research Institute of the TNRMС (Protocol No. 9 of 01.09.2020).

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Возможности радионуклидной диагностики Her2-позитивного рака молочной железы с использованием меченных технецием-99m таргетных молекул: первый опыт клинического применения

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РЕЗЮМЕ

Актуальность. Главной целью определения статуса Her2/neu в клинической практике прежде всего является измерение показаний для назначения таргетной терапии. Основными методами выявления статуса Her2/neu являются иммуногистохимический метод и флуоресцентная гибридизация *in situ* (FISH). Несмотря на распространенность, они имеют ряд существенных недостатков. В течение последних нескольких лет большое распространение приобретает радионуклидная диагностика с использованием нового класса альтернативных каркасных белков, отвечающих всем требованиям для оптимальной доставки радионуклида к опухолевым клеткам.

Цель. Изучение возможности клинического использования радиофармацевтического препарата на основе меченных технецием-99m адресных молекул для диагностики рака молочной железы с гиперэкспрессией Her2/neu у человека.

Материалы и методы. В исследование включены 11 больных раком молочной железы ($T_{1-4}N_{0-2}M_0$) до проведения системной терапии: пять человек с гиперэкспрессией Her2/neu; у шестерых экспрессия маркера выявлена не была. Во всех случаях выполнялось морфологическое и иммуногистохимическое исследование. При наличии значения Her2/neu 2+ проводился FISH-анализ. Препарат готовился непосредственно перед манипуляцией, после чего медленно внутривенно вводился пациенту. Сцинтиграфические исследования в режиме WholeBody и однофотонная эмиссионная компьютерная томография органов грудной клетки выполнялись через 2, 4, 6 и 24 ч после введения.

Результаты. Показатели радиохимического выхода, радиохимической чистоты и активности препарата непосредственно перед введением составили $(80 \pm 4)\%$, $(98 \pm 1)\%$ и $(434 \pm 19,5)$ МБк соответственно. Наибольший захват нормальными органами отмечался на временном отрезке в 6 ч в почках; умеренная активность – в печени и легких на том же временном промежутке. Изучение доз абсорбции препарата показало, что органом с наибольшей абсорбцией изучаемого соединения являлись почки; значительная аккумуляция определялась также в надпочечниках, желчном пузыре, печени, поджелудочной железе и селезенке. Наименьшее накопление изучаемого препарата отмечалось в головном мозге $(0,001 \pm 0,000)$ мГр и коже $(0,001 \pm 0,000)$ мГр. Эффективная доза при этом составила $(0,009 \pm 0,002)$ мГр. Различия между опухолями

с положительной и отрицательной экспрессией Her2-neu было выявлено на всех временных точках. Наилучший показатель при этом определялся через 2 ч инъекции препарата ($p < 0,05$).

Заключение. На основании полученных результатов можно свидетельствовать о том, что исследуемый радиофармацевтический препарат возможно рассматривать в качестве нового дополнительного метода диагностики Her2-позитивных опухолей молочной железы.

Ключевые слова: альтернативные каркасные белки, радионуклидная диагностика, Her2/neu, рак молочной железы, таргетная терапия.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Receptors of the epidermal growth factor family or EGFR (ErbB1/HER1, ErbB2/HER2, ErbB3/HER3, ErbB4/HER4) play an important role in the functioning of normal and tumor cells, responsible for the processes of cell division, differentiation, proliferation, migration, and apoptosis [1, 2]. The main attention of researchers is paid to the study of one member of the EGF family – the receptor for epidermal growth factor 2 (Her2/neu), the overexpression of which is detected in 15–20% of invasive breast cancer cases and is characterized by a poor prognosis and aggressive course of the tumor process [3, 4].

Establishing the Her2/neu status in clinical practice is primarily necessary to determine the indications for the appointment of targeted therapy using drugs, such as trastuzumab, pertuzumab, and trastuzumab emtansine in combination with chemotherapy or in mono-mode, which significantly improves survival rates in patients with overexpression of this marker [5, 6]. The main methods for diagnosing the Her2/neu status include immunohistochemical method and fluorescence *in situ* hybridization (FISH). Significant disadvantages of these techniques are the following: the impossibility of performing an *in vivo* study to determine the spread of a tumor process; the evaluation of cases associated

with heterogeneity of the Her2/neu receptor expression in tumor tissue; the need to perform invasive procedures (biopsy and (or) surgery), as well as possible differences in expression of the marker in the primary tumor and metastatic foci [7, 8].

The ongoing search for new effective agents has contributed to the development of molecular constructs that are alternative to the binding domains of antibodies and possess such characteristics as specific binding exclusively to the “target” antigen, lack of immunogenicity, stability, and the possibility of rapid chemical modification during research [9]. Over the past few years, a new class of alternative scaffold proteins (scaffolds) that meet all the requirements for optimal delivery of a radionuclide to tumor cells has become widespread [10]. The undoubted advantages of these constructs include significantly smaller sizes in comparison with the standard antibody, stable structure, additional functionalization and expression in the bacterial system, high thermal stability, and the possibility of direct chemical synthesis [11].

Currently, targeted radionuclide methods with high specificity for various molecular targets located on the surface of tumor cell membranes and making it possible to visualize foci of various sizes (the primary tumor node and metastatic foci) are becoming more widespread for the diagnosis of malignant tumors [12]. Until recently, monoclonal

antibodies (mAbs) were used as the main component of a radioimmunoconjugate [13].

However, the results of studies using mAbs did not meet the expectations assigned to them and revealed a number of features that significantly limit their use in clinical practice. Upon careful study, it turned out that mAbs have significantly reduced efficiency of interaction with the antigen, suboptimal pharmacological properties, slow distribution in the body, poor tissue penetration, and excretion by the kidneys (due to the high molecular weight of 150 kDa) [14]. The most significant disadvantage is the high immunogenicity of the used murine mAbs, which in response to their administration leads to the formation of neutralizing antibodies and, accordingly, to the occurrence of hyperimmune reactions and a decrease in the treatment effectiveness [15]. It became obvious that clinical use requires a radical modification of mAbs, including the correction of size, affinity, valence, etc. [16, 17].

One of the representatives of alternative scaffolds is ADAPT6, the albumin-binding domains of streptococcal protein G which are small in size (46–59 amino acid residues, molecular weight of 5–7 kDa). At the stage of preclinical studies, it was demonstrated that ADAPT6 labeled with various radionuclides shows a high contrast image of Her2-positive tumors in mouse xenografts just a few hours after injection [18, 19].

The aim of the present study was to investigate the possibility of clinical use of the ^{99m}Tc -ADAPT6 radiopharmaceutical for the diagnosis of breast cancer with overexpression of Her2/neu in humans.

MATERIALS AND METHODS

The clinical study was registered (ClinicalTrials.gov Identifier: NCT03991260) and approved by the Bioethical Committee of the Cancer Research Institute of TNRMC. It included 11 patients with breast cancer ($T_{1-4}N_{0-2}M_0$) before systemic chemotherapy or targeted therapy: in five people, Her2/neu overexpression was detected, and in six individuals, the expression of the marker was not detected. The average age of the patients was 50.7 ± 2.3 years. All patients signed a voluntary informed consent with information on the disclosure of the data received (clause 3 of article 13 of the Federal Law of the Russian Federation No. 323-FZ dated 21.11.2011).

The criteria for inclusion in the study were the following: newly diagnosed and morphologically verified breast cancer ($T_{1-4}N_{0-3}M_{0-1}$); the general condition of patients with 0–2 score according to the ECOG/WHO system; signed informed consent of the patient to participate in the research. Exclusion criteria were the presence of severe anemia, leukopenia, thrombocytopenia, sepsis, cachexia, severe concomitant pathology, claustrophobia, and refusal from treatment.

All patients were under dynamic observation for 48 hours after the administration of the radiopharmaceutical (RP) with an assessment of complaints, heart rate (HR), blood pressure (BP), and body temperature at various time intervals (before the administration of the drug, after 2, 4, 6, 24, and 48 hours after the injection). Additionally, all patients underwent laboratory tests (complete blood count and biochemistry and general urine analysis before the administration of the test compound, after 48 hours and 7 days) in the laboratory of the Cancer Research Institute of TNRMC.

Morphological research methods. In all cases, a morphological and immunohistochemical study of the biopsy material of the primary tumor was performed according to standard methods in the Laboratory of General and Molecular Pathology of the Cancer Research Institute, TNRMC. Diagnosis of breast cancer was established according to the “Histological classification of breast tumors” (World Health Organization, 2019). Immunohistochemical study of the biopsy material was carried out using antibodies from Dako (USA) to the oncoprotein c-erbB-2 (working dilution 1 : 500, rabbit). When evaluating the results, cases with no staining or with weak, intermittent membrane staining (categories 0 and 1+) were considered negative, and cases with strong staining of the entire cytoplasmic membrane of more than 10% of tumor cells (categories 3+) were positive. In the presence of weak to moderate staining of the entire cytoplasmic membrane of more than 10% of tumor cells (category 2+), all patients underwent FISH analysis.

FISH analysis was performed using an ERB-B2(17q12)/SE17 DNA probe (Kreatech, USA); the reaction result was evaluated using an Axiostar PLUS fluorescent microscope (Carl Zeiss, Germany). The test results were considered positive when the ratio of the average copy number of the

Her2/neu gene and the average number of chromosomes 17 centromeres in the cell was more than 2.2.

Radionuclide research methods. The drug was prepared immediately before administration in the Department of Radionuclide Diagnostics of the Cancer Research Institute of TNRMC according to the tricarbonyl method using the CRS Isolink kit (Center for Radiopharmaceutical Science, Paul Scherrer Institute, Villigen, Switzerland) [20].

To achieve the goals under aseptic conditions, the CRS Isolink kit (2.9 mg sodium tetraborate decahydrate, 7.8 mg sodium carbonate, 4.5 mg disodium boranocarbonate, and 9.0 mg potassium sodium tartrate tetrahydrate) was added with 500 μ l (2 GBq) of eluate $^{99m}\text{TcO}_4^-$ and incubated for 30 minutes at 100° C. Then, 500 μ l of tricarbonyl technetium was added to 500 μ g of ADAPT6 and incubated for 60 minutes at 50° C. Purification of the obtained compound from protein impurities and ADAPT6 molecules not bound to technetium was carried out using NAP-5 purification columns (GE Healthcare, Sweden). Radiochemical yield and purity were determined using thin-layer radiochromatography. Chromatogram analysis was performed using a Hitachi Chromaster HPLC systems chromatograph with a radioactive detector. The drug obtained after purification was diluted in 10 ml of sterile 0.9% NaCl solution, taken through a sterilizing filter and, after measuring the activity, was slowly injected into the patient intravenously.

Scintigraphy in WholeBody mode. Scintigraphic studies were performed on an E.CAM 180 gamma camera (Siemens, Germany) in the WholeBody mode using parallel high-resolution collimators for the energy of 140 keV in the supine position 2, 4, 6, and 24 hours after injection at a scanning speed of 12 cm / min.

Single-photon emission computed tomography was also performed in patients in the supine position 2, 4, 6, and 24 hours after drug administration. The field of view included the neck, axillary region, and chest to the level of tracheal bifurcation. 32 projections (each projection was 30 seconds long) were recorded into a 64 $\times$ 64 pixel matrix without hardware magnification.

Data processing and indicators used. During the study, the obtained data were subjected to post-processing using the specialized E. Soft software package (Siemens, Germany), while the level

of drug accumulation in the main organs and tissues was studied by tracing the region of interest (ROI) on the WholeBody images in front and rear projections. The RP biodistribution was presented as a percentage of its accumulation in the regions of interest to the total score in both projections.

The nature of the RP accumulation in the study area was also assessed: symmetry, intensity, uniformity of accumulation; the presence and number of focal inclusions of the indicator in the studied organ, regional lymph nodes (asymmetric areas of RP hyperfixation were considered pathological); the presence of other foci of pathological RP inclusion within the study area. In addition, in the study groups, a tumor/background quantitative indicator was calculated, reflecting the degree of drug accumulation in the pathological focus in comparison with intact tissues. The indicator was assessed by tracing the ROI of the tumor on axial slices with the best visualization of the latter; symmetric regions of interest of the contralateral mammary gland were used as a “background”.

Statistical processing of the results was carried out using the Statistica 10.0 for Windows software package and the nonparametric Mann – Whitney method. The difference between the two compared values was considered significant if the probability of their identity was less than 5% ($p < 0.05$). To calculate the RP absorption dose, the OLINDA/EXM 1.1 software was used with the application of an “adult woman” model.

RESULT AND DISCUSSION

According to the analysis, the indicators of radiochemical yield, radiochemical purity, and activity of the drug immediately before administration were (80 ± 4)%, (98 ± 1)%, and (434 ± 19.5) MBq, respectively. During the study, as well as during the observation period of the patients, no complaints, changes in HR, BP or temperature were detected. Changes in blood and urine parameters were also not detected.

The greatest uptake by normal organs was observed at a time interval of 6 hours in the kidneys ($(32 \pm 9)\%$ / ID / organ). Moderate activity of the compound was observed in the liver ($(2.6 \pm 0.8)\%$ / ID / organ) and lungs ($(2.0 \pm 0.6)\%$ / ID / organ) at the same time interval; the smallest uptake was in the small intestine at two hours after administration ($(0.9 \pm 0.3)\%$ / ID / organ). The results are shown in Fig. 1 and in Table 1.

Table 1

The greatest uptake of ^{99m}Tc by normal organs on planar scintigraphy after ^{99m}Tc -ADAPT6 injection, % / ID / organ				
Organ	Time period after injection			
	2 h	4 h	6 h	24 h
Kidneys	27 ± 10	31 ± 12	32 ± 9	29 ± 10
Lungs	3.3 ± 0.8	2.5 ± 0.8	2.0 ± 0.6	1.4 ± 0.8
Liver	3.2 ± 1.1	2.2 ± 1.1	2.6 ± 0.8	2.4 ± 1.0
Small intestine	0.8 ± 0.3	0.9 ± 0.3	0.8 ± 0.3	0.6 ± 0.2

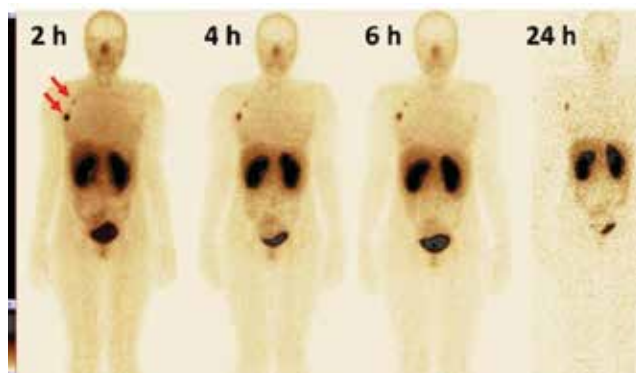


Fig. 1. Distribution of the ^{99m}Tc -ADAPT6 drug in organs and tissues of a breast cancer patient with positive Her2/neu expression 2, 4, 6, and 24 hours after injection: anterior and posterior views (arrows indicate the tumor)

The study of the absorption doses of the ^{99m}Tc -ADAPT6 drug showed that the organ with the highest absorption of the studied compound was the kidneys, (0.135 ± 0.42) mGy. Significant accumulation was also determined in the adrenal glands (0.023 ± 0.005) mGy, gall bladder (0.013 ± 0.008) mGy, liver (0.011 ± 0.008) mGy, pancreas (0.011 ± 0.008) mGy, and spleen (0.011 ± 0.008) mGy. The smallest accumulation of the studied drug was observed in the brain (0.001 ± 0.000) mGy and skin (0.001 ± 0.000) mGy. The effective dose was (0.009 ± 0.002) mGy (Table 2).

Table 2

Distribution of ^{99m}Tc -ADAPT6 in organs and tissues after administration in breast cancer patients	
Index	Absorption dose, mGy
Adrenal glands	0.023 ± 0.005
Brain	0.001 ± 0.000
Breast	0.007 ± 0.002
Gall bladder	0.013 ± 0.008
Lower colon wall	0.005 ± 0.001

Table 2 (continued)

Index	Absorption dose, mGy
Small intestine	0.006 ± 0.001
Stomach	0.006 ± 0.001
Upper colon wall	0.007 ± 0.001
Heart	0.004 ± 0.001
Kidneys	0.135 ± 0.42
Liver	0.011 ± 0.008
Lungs	0.005 ± 0.001
Ovaries	0.008 ± 0.002
Pancreas	0.011 ± 0.002
Muscle	0.003 ± 0.000
Red bone marrow	0.004 ± 0.001
Osteogenic cells	0.006 ± 0.001
Skin	0.001 ± 0.000
Spleen	0.011 ± 0.003
Thymus	0.005 ± 0.002
Thyroid	0.009 ± 0.004
Bladder	0.012 ± 0.007
Uterus	0.005 ± 0.001
Whole body	0.004 ± 0.001
Equivalent effective dose, mSv / MBq	0.017 ± 0.004
Effective dose, mSv / MBq	0.009 ± 0.002

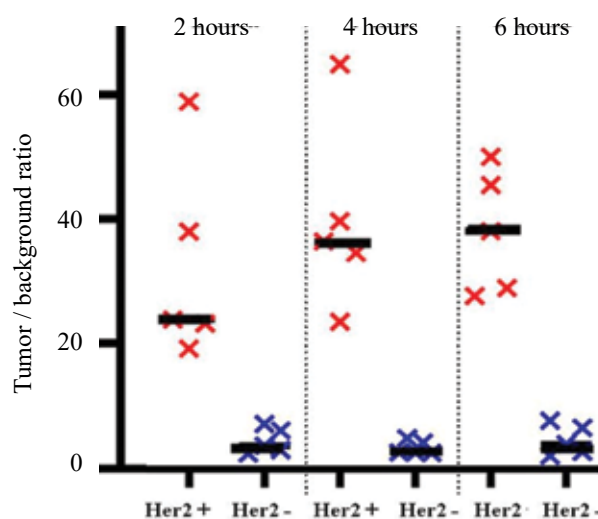


Fig. 2. Tumor / background ratio in patients with positive and negative expression of Her2/neu at different time points after drug administration

The difference between tumors with positive and negative expression of Her2-neu (comparison with the “background” site in the contralateral breast) after intravenous administration of ^{99m}Tc -ADAPT6 was detected at all time points. In this case, the best

indicator was determined 2 hours after the drug injection ($p < 0.05$) (Fig. 2).

CONCLUSION

This study has demonstrated the safety of clinical use, as well as good tolerance of ^{99m}Tc -ADAPT6 in breast cancer patients. The most significant results were found on drug accumulation in tumor tissue in patients with different Her2/neu expression. Thus, the revealed differences between Her2-positive and Her2-negative tumors ($p < 0.001$) will contribute to the optimization of approaches to the prescription of targeted drugs in oncological practice in the future. The accumulation of ^{99m}Tc -ADAPT6 in tumors with negative expression of the Her2/neu receptor is explained by the presence of at least 500 thousand receptors on the surface of the tumor cell, while with a positive status of this molecular parameter, its expression is up to 10 million receptors per cell. Similar phenomena are also observed when using radiopharmaceuticals, the targeting modules of which are representatives of other classes of target molecules [21].

Thus, based on the results obtained, it was concluded that the ^{99m}Tc -ADAPT6 radiopharmaceutical can be considered as a new additional method for diagnosing Her2-positive breast tumors.

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