Experimental Study of ^{99m}Tc-Aluminum Oxide Use for Sentinel Lymph Nodes Detection

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Abstract. The purpose of the study was a comparative research in the possibility of using the radiopharmaceuticals ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis for visualizing sentinel lymph nodes. The measurement of the sizes of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis colloidal particles was performed in seven series of radiopharmaceuticals. The pharmacokinetics of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis was researched on 50 white male rats. The possibility of the use of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis for lymphoscintigraphy was studied in the experiments on 12 white male rats. The average dynamic diameter of the sol particle was 52–77 nm for ^{99m}Tc-Al_2O_3 and 16.7–24.5 nm for ^{99m}Tc-Nanocis. Radiopharmaceuticals accumulated in the inguinal lymph node in 1 hour after administration; the average uptake of ^{99m}Tc-Al_2O_3 was 8.6% in it, and the accumulation of ^{99m}Tc-Nanocis was significantly lower—1.8% ($p < 0.05$). In all study points the average uptake of ^{99m}Tc-Al_2O_3 in the lymph node was significantly higher than ^{99m}Tc-Nanocis accumulation. The results of dynamic scintigraphic studies in rats showed that ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis actively accumulated into the lymphatic system. By using ^{99m}Tc-Al_2O_3 inguinal lymph node was determined in 5 minutes after injection and clearly visualized in all the animals in the 15th minute, when the accumulation became more than 1% of the administered dose. Further observation indicated that the ^{99m}Tc-Al_2O_3 accumulation reached a plateau in a lymph node (average 10.5%) during 2-hour study and then its accumulation remained practically at the same level, slightly increasing to 12% in 24 hours. In case of ^{99m}Tc-Nanocis inguinal lymph node was visualized in all animals for 15 min when it was accumulated on the average 1.03% of the administered dose. Plateau of ^{99m}Tc-Nanocis accumulation in the lymph node (average 2.05%) occurred after 2 hours of the study and remained almost on the same level (in average 2.3%) for 24 hours. Thus, the experimental study of a new domestic radiopharmaceutical showed that the ^{99m}Tc-Al_2O_3 accumulates actively in the lymph nodes several times as compared to the imported analogue and its practical application will facilitate intraoperative identification of sentinel lymph nodes.

INTRODUCTION

In recent years radionuclide techniques have proven to identify the sentinel lymph nodes (SLN) which are the first lymph nodes on the path of lymph drainage from a malignant tumor. These nodes, filtering afferent lymph, become a “trap” for cancer cells, and that is why SLN biopsy followed by histological examination is an objective diagnostic criterion for the lymphatic metastasis detection. It is believed that if the SLN are not affected by metastatic disease all other regional lymph nodes remain intact [1–7].

The world practice has considerable experience in relation to radionuclide visualization of SLN at melanoma and breast cancer. For the tumors in other locations (lungs, head, neck, gastrointestinal tract and uterus) the effectiveness of this method is studied in scientific research [1–3, 6].

Optimal radiopharmaceuticals to identify SLN is the colloid labeled with technetium-99m [1, 6]. In Russian Federation to identify the SLN colloidal rhenium sulphide (99mTc-Nanocis, CIS bio International) has been applied. The main disadvantage of this radiopharmaceutical is the low level of accumulation in the SLN (1.5–2% of the

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administered dose) and redistribution to lymph nodes of the 2nd and 3rd orders, which reduces the specificity of SLN visualization [1, 3, 6, 7].

Currently, in the Russian Federation there are no registered radiopharmaceuticals for SLN imaging. In this regard, the Tomsk Cancer Research Institute and Tomsk Polytechnic University developed the original radiopharmaceutical based on aluminum oxide (^{99m}Tc-Al_2O_3) labeled with technetium-99m (the project No. 16.N08.12.1011 "Preclinical studies of new lymphotropic radiopharmaceutical labeled by technetium-99m aluminum oxide" Federal Program "Development of the Russian Federation, the pharmaceutical and medical industry period up to 2020 and beyond").

The aim of present investigation was a comparative study of the possibility of using the radiopharmaceuticals ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis for visualizing sentinel lymph nodes in the experiment.

MATERIAL AND METHODS

The measurement of the sizes of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis colloidal particles was performed in seven series of radiopharmaceuticals on apparatus NICOMP 380 ZLS (company PSS NICOMP, USA).

The pharmacokinetics of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis was researched on white male rats "Wistar" weighing 200–250 g; the radiopharmaceuticals were injected between the first and second fingers of the hind paw subcutaneously in a dose of 30 MBq. Before the injection of radiotracer the animals were anesthetized with ethyl ether. The volume of the injected dose was 0.1 ml (volume activity is 300 MBq/ml). The animals were decapitated in groups of 10 individuals (5 rats for each radiopharmaceutical) in 1, 2, 3, 5 and 24 h after injection of the radiopharmaceuticals.

The recovered organs and tissues were packed into vials for weighing and direct radioactivity measurement. Standard radiopharmaceutical and bioassays have the same volume (after weighing vials filled with distilled water up to the same level) and the geometric form. The radioassay of blood and internal organs (the contents of the radiopharmaceutical as a percentage of the entered radioactivity per 1 ml of blood or 1 g of tissue or organ) was performed on the radiometer RIS A1 (Russian Federation). For radiometric studies a differential discriminator was set up on the photon peak of 140 keV, with a window width of 20%. According to the results of the radioassay the radiopharmaceutical level in inguinal lymph node and injection site was also determined.

The possibility of the use of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis for lymphoscintigraphy was studied in the experiments on 12 white male rats "Wistar" (6 rats for each radiopharmaceutical) weighing 300–350 g. Before a single subcutaneous injection of radiopharmaceuticals between the first and second fingers of the hind paw at a dose of 18–20 MBq and during scintigraphic studies the animals were anesthetized with ethyl ether. All scintigraphic studies were performed on the gamma-camera E-CAM-180 (Siemens, USA) with a setting up of a differential discriminator on a photon peak of 140 keV, with a window width of 20%, using a parallel low-energy high-resolution collimator. During the study the animals were put with their ventral surface to the detector of the gamma camera so that the whole body of the animal appeared in the picture. Since the introduction, kinetics of the distribution of radiopharmaceuticals through the organs and tissues was fixed by step-frame recording within 15 min (1 frame per minute), in the matrix of 64×64 pixels. Static scintigraphy was performed in 1, 2, 3 and 24 hours in the front and rear projections in the matrix of 256×256 with a set of 500 pulses per position. According to the results of the scintigraphic studies the percent of radiopharmaceuticals accumulation in the inguinal lymph node and the injection site from administered dose were determined.

Keeping and participation of the animals in the experiment were performed in accordance with the rules adopted by the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986).

The data received were processed by the method of variation statistics using "STATGRAF" numeric package. Between-group comparisons were done by using the Wilcoxon rank-sum test.

RESULTS AND DISCUSSION

The measurement of the sizes of radioactive particles of ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis in 7 batches showed that the average dynamic diameter of the sol particle was 52–77 nm for ^{99m}Tc-Al_2O_3 and 16.7–24.5 nm for ^{99m}Tc-Nanocis, which, according to the literature, is quite acceptable for scintigraphic visualization of lymph nodes [1, 3, 6].

TABLE 1. The content of 99mTc in the organs and tissues of rats at different times after subcutaneous injection of radiotracer ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis according to direct radiometry

Time		1 h	2 h	3 h	5 h	24 h
			^{99m}Tc-Al_2O_3			
Injection site	(%)	68.3 ± 5.3	57.0 ± 8.7	53.6 ± 5.0	51.1 ± 5.2	46.7 ± 6.7
Lymph node	%	8.6 ± 1.1*	10.5 ± 1.6*	11.2 ± 1.6*	11.8 ± 1.9*	12.8 ± 1.4*
Blood	%/ml	0.91 ± 0.01	0.26 ± 0.08	0.28 ± 0.08	0.30 ± 0.07	0.31 ± 0.08+
Liver	%	0.33 ± 0.15	0.38 ± 0.11	0.45 ± 0.14	0.43 ± 0.12	0.35 ± 0.11
	%/g	0.13 ± 0.06	0.15 ± 0.05	0.18 ± 0.06	0.18 ± 0.05	0.14 ± 0.07
Stomach	%	3.8 ± 1.2	9.6 ± 1.1	12.1 ± 2.4	16.8 ± 1.8	21.1 ± 1.6
	%/g	0.32 ± 0.1	0.8 ± 0.09	1.0 ± 0.14	1.4 ± 0.15	1.8 ± 0.14
Spleen	%	1.5 ± 0.4	2.6 ± 0.6	4.1 ± 0.9	4.3 ± 0.8	4.5 ± 0.9
	%/g	0.74 ± 0.2	1.4 ± 0.29	2.1 ± 0.43	2.2 ± 0.4	2.2 ± 0.42
Heart	%	0.41 ± 0.11	0.29 ± 0.12	0.67 ± 1.08	0.6 ± 0.07	0.9 ± 0.1
	%/g	0.34 ± 0.1	0.24 ± 0.09	0.56 ± 0.11	0.61 ± 0.06	0.76 ± 0.07
Lung	%	0.34 ± 0.12	0.26 ± 0.09	0.15 ± 0.06	0.13 ± 0.02	0.1 ± 0.08
	%/g	0.12 ± 0.05	0.09 ± 0.04	0.06 ± 0.03	0.05 ± 0.04	0.04 ± 0.3
			^{99m}Tc-Nanocis			
Injection site	(%)	66.3 ± 3.15	59.0 ± 4.1	55.5 ± 5.7	53.2 ± 4.9	51.2 ± 4.3
Lymph node	%	1.8 ± 1.2	3.6 ± 2.4	3.6 ± 1.8	3.6 ± 1.7	3.2 ± 1.0
Blood	%/ml	0.2 ± 0.1	0.24 ± 0.04	0.27 ± 0.03	0.27 ± 0.07	0.3 ± 0.1
Liver	%	1.2 ± 0.2	1.5 ± 0.2	1.5 ± 0.4	1.5 ± 0.7	2.2 ± 0.4
	%/g	0.48 ± 0.1	0.60 ± 0.15	0.62 ± 0.22	0.63 ± 0.31	0.88 ± 0.38
Stomach	%	6.6 ± 0.3	10.5 ± 0.9	13.9 ± 2.07	16.3 ± 1.9	18.3 ± 1.4
	%/g	0.55 ± 0.2	0.87 ± 0.21	1.15 ± 0.24	1.35 ± 0.31	1.52 ± 0.29
Spleen	%	1.3 ± 0.2	2.1 ± 0.3	2.8 ± 0.5	3.3 ± 0.7	3.6 ± 0.6
	%/g	0.65 ± 0.1	1.1 ± 0.15	1.4 ± 0.14	1.65 ± 0.2	1.80 ± 0.2
Heart	%	0.3 ± 0.1	0.3 ± 0.06	0.31 ± 0.09	0.3 ± 0.11	0.4 ± 0.15
	%/g	0.25 ± 0.09	0.24 ± 0.08	0.25 ± 0.08	0.24 ± 0.06	0.23 ± 0.07
Lung	%	0.6 ± 0.1	0.6 ± 0.12	0.61 ± 0.15	0.59 ± 0.14	0.54 ± 0.2
	%/g	0.22 ± 0.09	0.22 ± 0.1	0.23 ± 0.08	0.21 ± 0.06	0.20 ± 0.1

* $p < 0.05$% compared to ^{99m}Tc-Nanocis.

The radiometry of rats' organs showed that 99mTc-Al_2O_3 and 99mTc-Nanocis actively withdrew subcutaneous injection spot—there was about 2/3 of the administered dose in the spot after 1 hour (Table 1). About half of the dose remained in the injection site after 24 hours. Leaving the injection site, the radiopharmaceuticals were accumulated in the inguinal lymph node since 1 hour after administration the average accumulation of 99mTc-Al2O3 was 8.6% in it. The accumulation of 99mTc-Nanocis was significantly lower—1.8% ($p < 0.05$). During the second hour of study the average accumulation of 99mTc-Al_2O_3 in inguinal lymph node exceeded 10%, and in a day it increased gradually to 12.8%.

Table 2. The content of ^{99m}Tc in the injection site and inguinal lymph node after subcutaneous injection of radiotracer ^{99m}Tc-Al_2O_3 and ^{99m}Tc-Nanocis according to dynamic scintigraphy

Localization	Time after the injection	Average accumulation of ^{99m}Tc-Al_2O_3, %	Average accumulation of ^{99m}Tc-Nanocis, %
Injection site	5 min	76.8 ± 3.7	82.7 ± 5.0
	15 min	71.3 ± 4.2	67.7 ± 3.0
	1 h	68.2 ± 5.3	55.7 ± 4.8
	2 h	57.0 ± 8.6	50.5 ± 5.1
	3 h	53.5 ± 4.9	47.7 ± 4.6
	24 h	46.7 ± 6.8	35.33 ± 3.3
Inguinal lymph node	5 min	0.9 ± 0.2	0.98 ± 0.22
	15 min	1.19 ± 0.13	1.03 ± 0.22
	1 h	8.6 ± 1.1	1.4 ± 0.86
	2 h	10.5 ± 1.6	2.05 ± 1.67
	3 h	11.1 ± 1.2	2.18 ± 1.57
	24 h	12.0 ± 1.4	2.31 ± 1.45

* $p < 0.05$% compared to ^{99m}Tc-Nanocis.

The accumulation of 99mTc-Nanocis after 2 hours reached 3.6%, and fluctuated slightly at this level up to 24 hours of observation (Table 1). In all study points the average accumulation of 99mTc-Al_2O_3 in the lymph node was significantly higher than 99mTc-Nanocis accumulation.

Received through the thoracic duct into the blood radiopharmaceuticals were very actively taken up by liver and spleen. The hepatic level of the accumulation of 99mTc-Al_2O_3 rose from 3.8% in 1 h after injection to 21.1% in 24 hours of the study, for 99mTc-Nanocis these values were 6.6% and 18.3%, respectively. In the spleen, the magnitude accumulation of 99mTc-Al_2O_3 ranged from 1.5% (in 1 hour) to 4.5% (in 24 hours), the level of accumulation of 99mTc-Nanocis grew from 1.3% (in 1 hour) to 3.6% (in 24 hours). A slight content of radiopharmaceuticals in the heart, lungs and blood was noted, which recorded less than 1% of the injected dose.

The results of the dynamic scintigraphic studies in rats showed that 99mTc-Al_2O_3 and 99mTc-Nanocis accumulated actively in the lymphatic system from the injection site (Table 2). After 2 hours 57% of the administered dose of 99mTc-Al_2O_3 and 50.5% of 99mTc-Nanocis was in the injection site. After 24 hours less than half the dose of the radiopharmaceuticals was in the injection spot.

By using 99mTc-Al_2O_3 the inguinal lymph node was determined in 5 min after injection and clearly visualized in all the animals at the 15th min, when the accumulation became more than 1% of the administered dose. Further observation indicated that the 99mTc-Al_2O_3 accumulation reached a plateau in a lymph node (10.5% on average) for 2-hour study (Fig. 1), and then its accumulation remained practically at the same level, slightly increasing to 12% in 24 hours. In the case of 99mTc-Nanocis the inguinal lymph node visualized in all the animals in 15 min when it was accumulated on the average 1.03% of the administered dose.

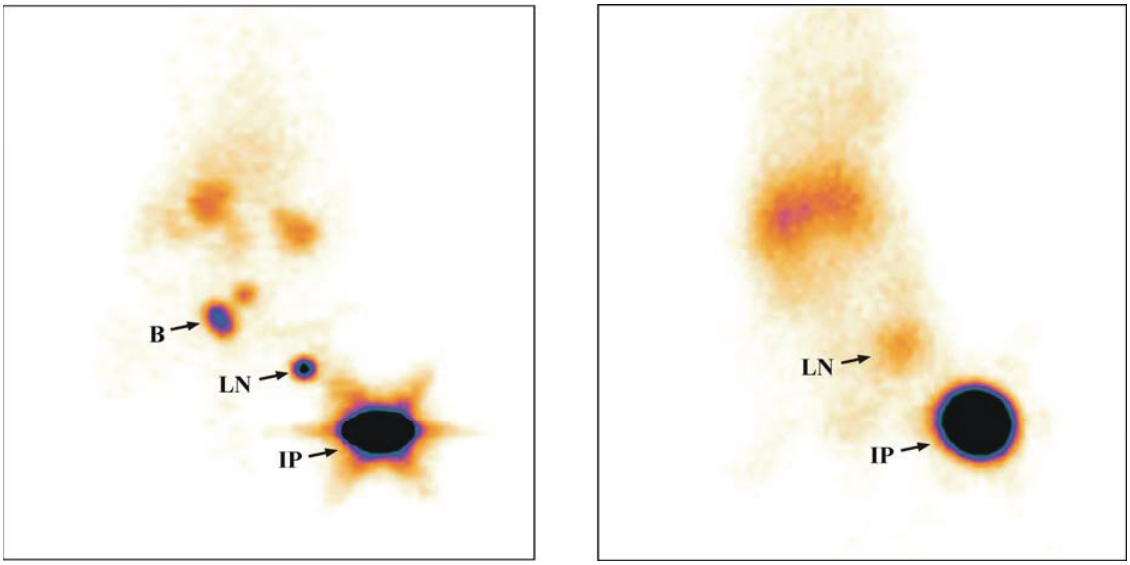
The plateau of 99mTc-Nanocis accumulation in the lymph node (2.05% on average) occurred in 2 hours of the study (Fig. 1) and remained almost at the same level (2.3% on average) to 24 hours.

In oncology practice the spots of radiopharmaceutical injections are often located in close proximity to the sentinel lymph node, making it difficult to visualize [1, 6].

We have established a multiple higher accumulation of 99mTc-Al_2O_3 in SLM in comparison with the import analogue. Considering this fact the clinical use of new domestic radiopharmaceutical will facilitate intraoperative identification of such nodes.

CONCLUSIONS

Thus, the experimental study of a new domestic radiopharmaceutical showed that the 99mTc-Al_2O_3 accumulates several times more actively in the lymph nodes as compared to the imported analogue and its practical application will facilitate intraoperative identification of SLN.



(a) (b)

FIGURE 1. Scintigrams rats performed through 2 hours after injection of ^{99m}Tc-Al_2O_3 (a) and ^{99m}Tc-Nanocis (b). A—The level of radioactivity in the injection point (IP)—56% of the injected dose, in the inguinal lymph node (LN)—11%. B—bladder. B—The level of radioactivity in the injection point (IP)—61.3% of the injected dose, in the inguinal lymph node (LN)—3.5%

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The study reported in this article was conducted according to accepted ethical guidelines involving research in humans and/or animals and was approved by an appropriate institution or national research organization. The study is compliant with the ethical standards as currently outlined in the Declaration of Helsinki. All individual participants discussed in this study, or for whom any identifying information or image has been presented, have freely given their informed written consent for such information and/or image to be included in the published article.

REFERENCES

1. V. I. Chernov, I. G. Sinilkin, E. Ch. Choynzonov, S. Y. Chijevskaya, A. A. Titskaya, R. V. Zelchan, O. D. Bragina, A. Y. Lyapunov, and V. S. Skuridin, Comparative evaluation of 99mTc-Al2O3 and 99mTc-fitat nanocolloids for sentinel lymph nodes visualization in patients with cancer of larynx and hypopharynx P 758, EANM Congress, October 10–14 2015, Hamburg, Germany, Eur. J. Nucl. Med. Mol. Imaging **42**(Suppl. 1), 704 (2015).
2. V. I. Chernov, I. G. Sinilkin, and S. V. Shiryaev, Radionuclide detection of sentinel lymph nodes, in *National Leadership on Nuclear Medicine*, edited by Yu. B. Lishmanova and V. I. Chernova (STT, Tomsk, 2010), pp. 336–343.
3. S. G. Afanas'ev, A. V. Avgustinovich, V. I. Chernov, and I. G. Sinilkin, Radioguided sentinel lymph node detection in gastric cancer patients, Siberian J. Oncology **4**, 27–32 (2009).
4. I. R. Jimenez, M. Roca, E. Vega, M. L. García, A. Benitez, M. Bajén, et al., Particle sizes of colloids to be used in sentinel lymph node radio localization, Nucl. Med. Commun. **29**, 166–172 (2008).
5. P. Paredes, Clinical relevance of sentinel lymph node in the internal mammary chain in Breast cancer patients, Q. J. Nucl. Med. **32**(11), 1283–1287 (2005).
6. A. J. Schauer, et al., *The Sentinel Lymph Node Concept* (Springer, Berlin, 2005).
7. I. Sinilkin, V. Chernov, R. Zelchan, A. Titskaya, and V. Skuridin, Clinical investigaition of nanocolloid 99m-Al2O3 for sentinel lymph nodes visualization, Congress of the European Association of Nuclear Medicine, Gothenburg, Sweden 18–22 Oct. 2014, Eur. J. Nucl. Med. Mol. Imaging **41**(Suppl. 2), 518 (2014).