Original Article
Mol Imaging Radionucl Ther 2023;32:28-34 DOI:10.4274/mirt.galenos.2022.77598

Leukocyte Labeling with Tc-99m-HMPAO: The Role of Leucocyte Numbers and Medication on the Labeling Efficacy and Image Quality

Tc-99m-HMPAO İşaretli Lökosit: Lökosit Sayısı ve İlaç Kullanımının Radyoişaretleme Verimi ve Görüntü Kalitesine Etkisi

Emre Karayel[1,2], Meltem Ocak[3], A. Seher Birteksöz Tan[4]

[1]*İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Nuclear Medicine, İstanbul, Turkey*
[2]*İstanbul University, Institute of Graduate Studies in Health Sciences, İstanbul, Turkey*
[3]*İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Turkey*
[4]*İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, İstanbul, Turkey*

Abstract

Objectives: The aim of this study is to evaluation of Tc-99m-hexamethylpropyleneamineoxime (HMPAO)-labeled leukocytes in terms of radiochemical, biochemical, and microbiological quality controls and to examine the effect of leukocyte numbers of the blood obtained from patients and the medications currently used by the patients on the radiochemical yields of Tc-99m-HMPAO-labeled leukocytes, and imaging quality was evaluated.

Methods: Thirty paients were included in our study who applied to Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Nuclear Medicine for Tc-99m-HMPAO-labeled leukocyte scintigraphy. Devices and chemicals used in the preparation of Tc-99m-HMPAO-labeled laukocytes were compared with other nuclear medicine clinics. Tc-99m-HMPAO-labeled leukocytes were evaluated in terms of radiochemical, biochemical, and microbiological quality controls. The effect of leukocyte numbers of the blood obtained from patients and the medications currently used by the patients on the radiochemical yields of Tc-99m-HMPAO-labeled leukocytes and imaging quality was evaluated.

Results: The pH range of Tc-99m-HMPAO was 6-8 and the radiochemical purity was 90±2.04% (n=30), the radiochemical yield of Tc-99m-HMPAO-labeled leukocytes was 51±2.18% (n=30), the radiolabeling yield of Tc-99m-HMPAO-labeled leukocyte increased as the amount of white blood cell in the blood increased and whether the patients used any antibiotic, blood thinners, insulin and blood pressure medications did not affect the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes. The number of erythrocytes were removed at a rate of >99% in LPR by starch solution (6% HES; in the hemocytometric examination of Tc-99m-HMPAO-labeled leukocytes performed zeroth and 4th h, living/dead cell ratio was found 97.5% and the product was sterile.

Conclusion: Tc-99m-HMPAO was labeled with leukocytes successfully, and Tc-99m-HMPAO-labeled leukocytes was safely injected to the patients as sterile without loss of vitality and aggregation.

Keywords: Tc-99m-HMPAO, Tc-99mHMPAO-labeled leukocytes, infection imaging

Öz

Amaç: Bu çalışmada; Tc-99m-heksametilpropilenaminoksim (HMPAO) ile işaretli lökositlerin radyokimyasal, biyokimyasal ve mikrobiyolojik açıdan kalite kontrollerinin incelenmesi ve hasta kanının ve lökosit süspansiyonunun ihtiva ettiği lökosit miktarı ile hastaların kullandıkları bazı ilaçların Tc-99m-HMPAO ile lökositlerin radyoişaretleme verimine etkisinin incelenmesi amaçlanmıştır.

Address for Correspondence: Prof. Emre Karayel MD, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Nuclear Medicine; İstanbul University, Institute of Graduate Studies in Health Sciences, İstanbul, Turkey İstanbul, Turkey
Phone: +90 212 440 00 00 **E-mail:** emre.karayel@iuc.edu.tr ORCID ID: https://orcid.org/0000-0001-8114-2315
Received: 26.05.2022 **Accepted:** 27.07.2022

Yöntem: Çalışmaya İstanbul Üniversitesi-Cerahhapaşa, Cerrahpaşa Tıp Fakültesi, Nükleer Tıp Anabilim Dalı'na Tc-99m-HMPAO ile işaretli lökosit sintigrafisi için başvuran 30 hasta dahil edildi. Radyoişaretleme sırasında kullandığımız alet ve kimyasallar literatürde belirtilenlerle diğer örneklerle karşılaştırıldı. Hazırlanan radyofarmasötiğin kalite kontrolleri radyokimyasal, biyokimyasal ve mikrobiyolojik açıdan incelendi. Hasta kanının ve lökosit süspansiyonunun ihtiva ettiği lökosit miktarları ile hastaların kullandıkları antibiyotik, kan sulandırıcı, insülin ve tansiyon ilaçlarının Tc-99m-HMPAO ile lökositlerin radyoişaretleme verimine etkisi SPSS-20 programı ile istatistiksel olarak değerlendirildi.

Bulgular: Tc-99m-HMPAO radyofarmasötiğinin pH aralığının 6-8 ve radyokimyasal saflığının %90±2,04 (n=30), Tc-99m-HMPAO ile işaretli lökositlerin radyokimyasal veriminin %51±2,18 (n=30), hastaların antibiyotik, kan sulandırıcı, insülin ve tansiyon ilacı kullanma durumunun Tc-99m-HMPAO ile işaretli lökositlerin radyokimyasal verimine etki etmediği, hasta kanında ve lökosit süspansiyonunda bulunan lökosit miktarı arttıkça Tc-99m-HMPAO ile işaretli lökositlerin radyokimyasal veriminin de arttığı, kullanılan nişasta çözeltisinin (%6 HES) kırmızı kan hücrelerinin %99'dan fazlasını ortamdan uzaklaştırdığı, işaretli lökositlerin hemositometrik yöntemle 0 ve 4. saatte yapılan analizleri sonucu lökositlerin %97,5 oranında canlılığını koruduğu ve son ürünün steril olduğu tespit edildi.

Sonuç: Tc-99m-HMPAO ile lökositler başarılı bir şekilde işaretlenmiş ve hastalara steril, canlılığını kaybetmemiş ve pıhtılaşma olmayan Tc-99m-HMPAO ile işaretli lökositlerin güvenle uygulanabileceği sonucuna varılmıştır.

Anahtar kelimeler: Tc-99m-HMPAO, Tc-99m-HMPAO işaretli lökosit, enfeksiyon görüntüleme

Introduction

Radiopharmaceuticals are drugs used in nuclear medicine paired with radiation sources. Radiopharmaceuticals with alpha or beta emissions are widely used for cancer treatment, while gamma ray emitters are used in diagnostic imaging. In the recent years, hybrid imaging systems such as single photon emission tomography/computed tomography (SPECT/CT), positron emission tomography/CT, PET/magnetic resonance have added significant progress in the use of diagnostic radiopharmaceuticals (1,2,3,4,5). Technetium-99m (Tc-99m) methylene diphosphonic acid, ^{67}Gallium-citrate, Tc-99m-nanocolloid, Tc-99m-human immunoglobulin, ^{18}F-fluorodeoxyglucose, Tc-99m-hexamethylpropyleneamineoxime-leukocyte (HMPAO-leukocyte) are the most common radiopharmaceuticals used for infection imaging. Due to differences in the uptake mechanisms, these radiopharmaceuticals can be used alone or together to increase specificity and sensitivity. Tc-99m-HMPAO-labeled leukocyte scintigraphy is a frequently preferred imaging method in infection imaging with its high specificity and sensitivity and can be used to determine various disorders such as occult site of infection, osteomyelitis of the appendicular skeleton, infected joint and vascular prosthesis, diabetic foot, fever of unknown origin, postoperative abscesses, lung infections, endocarditis, inflammatory bowel disease, neurological infections, infected central venous catheters or other devices (6,7,8,9).

Preparing Tc-99m-HMPAO-labeled leukocytes requires considerable and careful efforts in terms of radiation protection, and the blood may be infected with the pathogens. Moreover, the quality of the administered radiopharmaceutical is quite significant for mainting patient safety after administration.

In this study, we examined Tc-99m-HMPAO-labeled leukocytes in a cohort of 30 patients who applied to Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Nuclear Medicine. The methods, devices and chemicals used in the preparation of Tc-99m-HMPAO-labeled laukocytes were compared with other nuclear medicine clinics in Europe. Additionally, it was assessed the possible effects of using different methods, devices and chemicals, the currently used medications by patients and the numbers of leukocytes [white blood cells (WBCs)] and erythrocytes [red blood cells (RBCs)].

Materials and Methods

Thirty paients were included in our study who applied for Tc-99m-HMPAO-labeled leukocyte scintigraphy (16 F, 14 M and age 62±15.1).

Primarily, patients were informed about Tc-99m-HMPAO-labeled leukocyte scintigraphy, and radiation protection instructions were provided. Patients' age, height, weight, gender, blood group, and history were noted before starting the procedure, in addition to whether they use certain medications such as antibiotics, insulin, blood pressure , and blood thinners.

The preparation of Tc-99m-HMPAO-labeled leukocytes was carried out under 2 main headings: "isolation of leukocytes", and radiolabeling and purification". All procedures were carried out under aseptic condition.

Isolation of Leukocytes

A total of 80 mL blood was taken from the patients with a butterfly needle (20 G) gently, 40 mL each into 2 sterile 50 mL syringes containing 0.5 mL anticoagulant (vasparin 1). 500 µL of 80 mL blood taken from the patients were separated to determine the number of leukocytes, erythrocytes, neutrophils, and lymphocytes in the blood. Samples with the number of WBCs >5.5 (10^3/uL) in the blood were compared with those with <5.5 (10^3/uL) through the change in the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes. 10 mL of 80 mL was

centrifuged (2000 g) and 3-5 mL volume of supernatant (cell free plasma, CFP) was used to dilute Tc-99m-HMPAO-labeled leukocytes just before the injection (6,10,11).

7 mL of PF polihes (HES 200/0.5) 6% starch solution was added to the remaining 50 mL syringes containing approximately 35 mL blood, and mixed slowly and left for erythrocyte sedimentation for 40-60 minutes. During sedimentation, the amount of supernatant (leukocyte-rich plasma, LRP) was visually checked. At the end of the sedimentation, the supernatant was transferred to a falcon tube before centrifuging (150 g, 5 min) 500 µL of the supernatant was separated again to determine the numbers of RBC and WBC in LRP by flow cytometry. Samples with the numbers of WBCs $>2\times10^8$ in the blood were compared with those with $<2\times10^8$ through the change in the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes. After centrifugation, the pellet was diluted with 2.5 mL vasparin-2 solution (6,10,12).

Vasparin-1 solution: 1.6 mL of heparin sodium (25.000 IU) + 8.4 mL NaCl (0.09%).

Vasparin-2 solution: 0.1 mL of vasparin-1 solution + 9.9 mL NaCl (0.09%).

Radiolabeling and Purification

Tc-99m-HMPAO was prepared by adding 40 mCi/2 mL freshly eluted (<30 min) Tc-99m to the HMPAO cold kit and incubated 10 min at room temperature. Afterward, leukocytes in 2.5 mL vasparin-2 solution and 20 mCi/1 mL Tc-99m-HMPAO were mixed into a 50 mL sterile falcon tube and incubated for 20 min at room temperature. During incubation, the mixture was shaken periodically. At the end of the incubation, the mixture was centrifuged (150 g, 5 min) and the supernatant was removed via pasteur pipette. Both pellet and supernatant were counted with a dose calibrator to calculate the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes (6,10,11).

Then, the pellet was diluted with 20 mL of sterile saline and 1 mL of 20 mL labeled leukocyte suspension separated for inoculation to sabouraud dextrose broth medium and 1 mL to fluid thioglycollate medium The remaining labeled leukocyte suspension was centrifuged over again (150 g, 5 min) and the supernatant was removed. The pellet was diluted with CFP. 100 µL of the patients' dose was separated for the hemocytometer method to determine the viability of leukocytes (6,10,11).

Quality Controls

Radiolabelling Efficiency of Tc-99m-HMPAO

The radiolabelling efficiency of Tc-99m-HMPAO was carried out with the paper chromatography method. Whatmann-3 paper was used as the stationary phase and ethyl acetate was used as the mobile phase (13,14). Lipo-Tc-99m-HMPAO showed an Rf value of 1.0. Hydro-Tc-99m-HMPAO and free Tc-99m showed an Rf value of 0.0. The strips were scanned with a scanner (Cyclone® Plus Storage Phosphor System, Perkin Elmer, Milan, Italy). The effect of the radiochemical purity on the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes was analyzed with SPSS-20.

Biochemical Analysis

The numbers of RBC, WBC, neutrophil and lymphocytes in the blood and numbers of RBC and WBC in LPR were determined by flow cytometry using a Beckman Coulter-LH780 device in a volume of 200 µL and manual method (15). The effect of the number of WBC in the blood and LPR on the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes was analyzed with SPSS-20.

Microbiological Quality Control of Tc-99m-HMPAO-labeled Leukocyte

1 mL samples obtained from 20 mL Tc-99m-HMPAO-labeled leukocytes, which diluted with 0.9% NaCl were kept until the amount of radioactivity fell below the limits allowed by the Nuclear Regulatory Authority, and then sterility test in accordance with European Pharmacopoeia was applied at Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology (6,16). Additionally, 100 µL samples obtained from the Tc-99m-HMPAO-labeled leukocyte were added to the Eppendorf tubes containing 100 µL methylene blue before the injection. The sample was shook gently and dripped onto a hemocytometry slide and examined under a light microscope to determine the viability of leukocytes at zeroth and 4th hours (6).

SPECT/CT Imaging Procedure and Calculation of Spleen/Liver Ratio

Scintigraphy images were acquired at 30 min and 4 h after Tc-99m-HMPAO injection. At 30 min, a static (planar) image was made for 10 min with 256x256 matrix size followed by a 1 minute spleen static image with the same matrix size. At the 4th h, static imaging was again conducted for up to 10 min with a 256x256 matrix, then SPECT/CT was performed with 32 frames, 45 second/frame, and 128x128 matrix size (17).

From 30 minutes images, spleen/liver uptake ratio, and lung uptake was derived and lung uptake was calculated at the 4th-hour via Image-J program (10.6 mCi, n=3).

This study Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Ethics Committee approval was obtained (number: 59491012-604.01.02), and all patients signed written informed consent.

Statistical Analysis

The effect of the blood thinners, insulin, antibiotics and blood pressure medications, and the amount of leukocytes in the blood and LPR were explored through the change in the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes using the SPSS-20 program. Mann-Whitney U test was used for comparing the means as the data showed no normal distribution.

Results

Isolation of Leukocytes

Any abnormalities, aggregates, clumps, or clots were not observed the during the isolation of leukocytes.

Quality Controls

Radiolabeling and Purification

The pH of Tc-99m-HMPAO was measured between 6 and 8 via a pH strip. The contents of the kit were completely dissolved and the radiochemical purity of Tc-99m-HMPAO was 90±2.04% (n=30) (Table 1).

During the radiolabeling and purification of Tc-99m-HMPAO-labeled leukocytes, it was observed that the leukocytes did not aggregate and the radiolabeling yield was found >40% for 28 patients, <40% for 2 patients, and 51±10.9% on average (Table 2).

Table 1. Radiochemical purity of Tc-99m-HMPAO

Patient no	The radiochemical purity of Tc-99m-HMPAO (%)	Patient no	The radiochemical purity of Tc-99m-HMPAO (%)
1	93.4	**16**	91
2	91	**17**	89.5
3	91	**18**	89.5
4	95.7	**19**	91
5	87	**20**	93
6	87	**21**	93
7	91	**22**	89
8	91	**23**	89
9	91	**24**	91
10	91	**25**	91
11	91	**26**	90
12	91	**27**	90
13	86.4	**28**	92
14	86.4	**29**	92
15	93.4	**30**	90

HMPAO: Hexamethylpropyleneamineoxime

Biochemical Analysis

The numbers of leukocytes, erythrocytes, neutrophils, and lymphocytes in the blood samples of patients and the numbers of erythrocytes in the LRP are shown in Table 3.

Microbiological Quality Control

No microbial growth was found in Tc-99m-HMPAO-labeled leukocytes, it was sterile. Additionally, in the hemocytometric examination of Tc-99m-HMPAO-labeled leukocytes performed zeroth and 4th hour, living/dead cell ratio was found 97.5%.

The Effect of the Medications Used by the Patients on the Radiolabeling Yield of Tc-99m-HMPAO-Labeled Leukocyte

The effect of whether the patients used any antibiotics, blood thinners, insülin, and blood pressure medications, as well as the quantity and duration of medications on the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes were analyzed with SPSS-20 (Table 4). It was revealed that the frequently used medications have no effect on the radiolabeling yield ($p>0.05$).

SPECT/CT Imaging

At SPECT/CT imaging, it was found that the spleen uptake was more than liver uptake and at 30th minute and 4th hour imaging there was no uptake in the lung.

Discussion

This study aimed to adapt the protocol specified as "Guidelines for the labeling of leucocytes with Tc-99m-HMPAO" (6) to our own conditions at Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Nuclear Medicine. Also, the evaluation of Tc-99m-HMPAO-labeled leukocytes in terms of radiochemical, biochemical, and microbiological quality control. Furthermore, the effect of the medications currently used by the patients and leukocyte numbers in the blood obtained from patients and LPR on radiochemical yields of Tc-99m-HMPAO-labeled leukocytes and imaging quality was evaluated.

Frequent medications involving antibiotics, insulin, blood pressure, and blood thinners as well as the amount and duration of use were recorded. In despite of these results, the medications currently used by the patients did not affect the imaging quality and the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes statically ($p>0.05$). In line with these findings, it was concluded that patients can safely use these medications before the procedure as this is a crucial issue that impacts the quality of life of patients.

During the leukocyte isolation step, the patient's blood was taken into 50 mL injectors containing vasparin-1

Table 2. Radiolabeling efficiency of Tc-99m-HMPAO-labeled leukocytes

No	Tc-99m-HMPAO activity (mCi)	Tc-99m-HMPAO-labeled leukocyte activity (mCi)	Radiolabeling efficiency (%)	No	Tc-99m-HMPAO activity (mCi)	Tc-99m-HMPAO-labeled leukocyte activity (mCi)	Radiolabeling efficiency (%)
1	20	10,5	52.5	**16**	20	8.25	41.25
2	20	9	45	**17**	20	13.01	65.05
3	20	10	50	**18**	20	10.4	52
4	20	10.3	51.5	**19**	20	14.8	74
5	20	8.75	43.75	**20**	20	12.4	62
6	20	12.6	63	**21**	20	10.5	52.5
7	20	9	45	**22**	20	8.1	40.5
8	20	11.38	56.9	**23**	20	10.55	52.75
9	20	10.35	51,75	**24**	20	7.2	36
10	20	12.00	60	**25**	20	13.83	69.15
11	20	9.69	48.45	**26**	20	8.61	43.05
12	20	15.2	76	**27**	20	11.2	56.15
13	20	9.6	48	**28**	20	9.2	46
14	20	8.5	42.5	**29**	20	9.9	49.5
15	20	10.1	50.5	**30**	20	5.1	25.5

HMPAO: Hexamethylpropyleneamineoxime

Table 3. Analysis of cell numbers of leukocytes, erythrocytes, neutrophils and lymphocytes

Patient no	RBCs in blood (10^6/uL)	RBCs in LRP (10^6/uL)	WBC (10^3/uL)	Neutrophils (%)	Lymphocytes (%)	Patient no	RBCs in blood (10^6/uL)	RBCs in LRP (10^6/uL)	WBC (10^3/uL)	Neutrophils (%)	Lymphocytes (%)
1	4.65	0.03	5.1	77.4	16.1	**16**	5.36	0.02	3.8	56	39
2	4.78	0.04	5.1	45.6	46.1	**17**	4.76	0.02	10.3	59	29
3	6.35	0.04	5.4	70.6	20.7	**18**	4.53	0.04	6.4	58	35
4	3.9	0.03	7	53.2	37.4	**19**	4.86	0.03	5.5	45.8	34.3
5	5.49	0.03	5.1	67	25.4	**20**	4.21	0.03	5	61.3	33.3
6	5.85	0.03	3.4	52.4	42.6	**21**	4.89	0.03	10	82.5	12.1
7	4.72	0.04	4.5	62.1	32.1	**22**	4.66	0.03	3.8	43.7	41
8	5.9	0.04	5.3	73.8	19	**23**	4.77	0.03	7.4	49.5	36.1
9	5.11	0.04	9.2	85.9	11.7	**24**	6.29	0.04	6.2	65.2	28.7
10	6.45	0.02	2.9	69.5	23.3	**25**	5.68	0.03	6.3	54	33.6
11	5.67	0.02	4	64.7	28.7	**26**	5.01	0.02	2.8	56	40.7
12	3.99	0.03	5.8	70	19.9	**27**	7.63	0.03	3.3	67.5	25.9
13	4.59	0.03	3.7	66.3	21.2	**28**	4.1	0.02	4.4	86.9	8.7
14	4.85	0.05	5.1	56.4	37.5	**29**	4.62	0.01	3.5	82.5	6
15	4.71	0.03	3.7	55.5	40	**30**	4.1	0.02	4.4	86.9	8.7

RBC: Red blood cell, WBC: White blood cell, LRP: Leukocyte-rich plasma

Table 4. Medications currently used by patients and radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes				
	Antibiotics	**Blood thinners**	**Insulin**	**Blood pressure**
Number of patients using medications	7	9	10	9
Radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes belongs patients using medications (mCi)	10.54	11.37	10.93	10.48
Number of patients not using medications	23	21	20	21
Radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes belongs patients not using medications (mCi)	10.27	9.89	10.03	10.27
HMPAO: Hexamethylpropyleneamineoxime				

solution to prevent coagulation. In the guidelines (6), anticoagulant citrate dextrose solution (ACD-A) is used as an anticoagulant. In our study, since ACD-A is not available in our department, heparin sodium was used instead of ACD-A, and any coagulation problem was not experienced.

In the guidelines (6), it is stated that at least 2×10^8 leukocytes must achieve a good labeling efficiency. In our study, the mean number of leukocytes in the blood and in the LPR was determined as 3.69±1.36x10^8 and 2.89.88±x10^8 respectively and these are above the numbers specified in the guidelines. The samples with the number of WBCs >5.5 (10^3/uL) in the blood were compared with those of <5.5 (10^3/uL). And the samples with the numbers of WBC >2×10^8 in the LPR were compared to those of <2×10^8. The radiochemical yield of the Tc-99m HMPAO-labeled leukocytes of the samples with the number of WBC >5.5 (10^3/uL) in the blood was higher than those of <5.5 (10^3/uL). The radiochemical yield of the Tc-99m HMPAO-labeled leukocytes of the samples with the number of WBC >2×10^8 in the LPR was higher than those of <2×10^8. For the first time in our study, the effect of the WBC numbers on the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes was compared.

According to guidelines (6), 10% HES (200/0.5 or 200/0.6) solution is recommended for getting LPR from the blood. In our study, we use 6% HES (200/0.5) solution, which was used by other groups (10,12). The erythrocyte numbers in the LPR determined with flow cytometer was found average 0.03±0.008*10^6/uL. In other words, the number of erythrocytes were removed at a rate of >99% in LPR. This result is similar to the other group (11).

It is relevant to prepare Tc-99m-HMPAO during radiolabeling as it affects the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes. In our study, radiochemical purity of Tc-99m-HMPAO prepared with freshly eluted Tc-99m-pertechnetate, an average of 90%±2.04 was found and it falls within the limits specified in European Pharmacopeia.

While lipo- Tc-99m-HMPAO is labeled with leukocytes, it passes into the cell by passive diffusion and becomes trapped there. In the guidelines (6), it is stated that >40% radiochemical yield is sufficient. In our study, the average radiolabeling yield of Tc-99m-HMPAO-leukocytes was found 51±10.9% and it was within the limits accepted by the guideline.

As with all other intravenously injected drugs, microbiological tests must be applied to Tc-99m-HMPAO-labeled leukocytes, which are classified as drugs by the Food and Drug Administration. In our study, a sterility test was applied to Tc-99m-HMPAO-labeled leukocytes in accordance with the European Pharmacopoeia. The samples were inoculated in liquid sabouraud medium and incubated for 14 days in an incubator at 25 °C and inoculated in thioglycolate liquid medium and incubated for 14 days in an incubator at 37 °C. Under normal conditions, at the end of the incubation period, the presence of growth in these media is evaluated according to the presence of turbidity with visual controls. However, since the samples contain leukocyte cells in both media, it is not possible to control the turbidity visually. At the end of the 14-day incubation period, the samples taken from the liquid sabouraud medium are cultured again into the sabouraud dextrose agar medium for 5 days, and the samples taken from the thioglycolate liquid medium are cultured again into the tryptic soybean medium for 3 days. At the end of the incubation periods, it was evaluated visually whether bacteria or fungal colonies grew on the media. Additionally, the samples were inoculated in sheep blood agar medium to grow other microorganisms and to determine hemolysis reactions, and were incubated for 3 days in an incubator at 37 °C. According to the sterility test, it was determined that there was no growth in all media and all the products were sterile.

Because of the visual controls, no clots or clusters were found. The results are similar to other studies (6,10,11).

While preparing Tc-99m-HMPAO-labeled leukocytes, the leukocytes must not lose their vitality during both labeling and after they are injected. Leukocytes that lose vitality are not labeled during the labeling process and the biodistribution is not like living cells after injection. In our study, the viability control leukocytes were performed with the hemocytometer and number of viable cells was found

97.5%. In the guidelines (6), it is stated that >96% viable cells are sufficient.

At SPECT/CT imaging, it was found that the spleen uptake was more than liver uptake and at 30th minute and 4th h scans, there was no uptake in the lung same as the guideline reported (6).

Conclusion

The protocol specified "Guidelines for the labeling of leucocytes with Tc-99m-HMPAO" (6) is successful and applicable at our site at Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Nuclear Medicine. Tc-99m-HMPAO-labeled leukocytes was safely injected as sterile without loss of vitality and aggregation .

It was found that the medications currently used by the patients did not affect the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes and the radiolabeling yield of Tc-99m-HMPAO-labeled leukocytes increased as the leukocyte numbers increased.

Consequently, the protocol of preparing Tc-99m-HMPAO-labeled leukocytes and quality controls was installed in our department.

Ethics

Ethics Committee Approval: Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Ethics Committee approval was obtained (number: 59491012-604.01.02).

Informed Consent: All patients signed written informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.K., M.O., A.S.B.T., Data Collection or Processing: E.K., M.O., A.S.B.T., Analysis or Interpretation: E.K., M.O., A.S.B.T., Literature Search: E.K., M.O., A.S.B.T., Writing: E.K., M.O., A.S.B.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This research was supported by Istanbul University Scientific Research Projects (BAP) (project number: 25322).

References

1. Vermeulen K, Vandamme M, Bormans G, Cleeren F. Design and challenges of radiopharmaceuticals. Semin Nucl Med 2019;49:339-356.
2. Demir M. Nükleer Tıp Fiziği ve Klinik Uygulamaları (4. baskı). İstanbul, 2014.
3. Brugarolas P, Comstock J, Dick DW, Ellmer T, Engle JW, Lapi SE, Liang SH, Parent EE, Kishore Pillarsetty NV, Selivanova S, Sun X, Vavere A, Scott PJH; Society of Nuclear Medicine and Molecular Imaging Radiopharmaceutical Sciences Council. Fifty years of radiopharmaceuticals. J Nucl Med Technol 2020;48(Suppl 1):34S-39S.
4. Papagiannopoulou D. Technetium-99m radiochemistry for pharmaceutical applications. J Labelled Comp Radiopharm 2017;60:502-520.
5. Uhl P, Fricker G, Haberkorn U, Mier W. Radionuclides in drug development. Drug Discov Today 2015;20:198-208.
6. de Vries EF, Roca M, Jamar F, Israel O, Signore A. Guidelines for the labelling of leucocytes with (99m)Tc-HMPAO. Inflammation/infection taskgroup of the European Association of Nuclear Medicine. Eur J Nucl Med Mol Imaging 2010;37:842-848. Erratum in: Eur J Nucl Med Mol Imaging 2010;37:1235.
7. Govaert GAM, Bosch P, IJpma FFA, Glauche J, Jutte PC, Lemans JVC, Wendt KW, Reininga IHF, Glaudemans AWJM. High diagnostic accuracy of white blood cell scintigraphy for fracture related infections: results of a large retrospective single-center study. Injury 2018;49:1085-1090.
8. Granados U, Fuster D, Soriano A, García S, Bori G, Martínez JC, Mayoral M, Perlaza P, Tomás X, Pons F. Screening with angiographic images prior to (99m)Tc-HMPAO labelled leukocyte scintigraphy in the diagnosis of periprosthetic infection. Rev Esp Med Nucl Imagen Mol 2015;34:219-224.
9. Pijarowska-Kruszyna J, Karczmarczyk U, Jaroń AW, Laszuk E, Radzik M, Garnuszek P, Mikołajczak R. New synthesis route of active substance d,l-HMPAO for preparation technetium Tc99m exametazime. Nucl Med Rev Cent East Eur 2017;20:88-94.
10. Martinez T, Gallego Peinado M, Sanchez Catalicio J, Perez Angel F, Contreras Gutiérrez J. Validation of the leukocyte labeling procedure with a new kit for the preparation of technetium-99m exametazime injection. Nucl Med Commun 2016;37:432-434.
11. Kim EM, Jeong HJ, Lim ST, Sohn MH. Analysis of cell fraction of 99mTc-HMPAO radiolabeled leukocytes. Curr Radiopharm 2020;13:142-148.
12. Hung JC, Chowdhury S, Mullan BP. Use of stabilized technetium-99m-exametazime for radiolabeling leukocytes. J Nucl Med 1998;39:912-917.
13. Taşçı C, Ülker Ö, Ertay T, Taner MS, Soylu A, Berk F, Ünak P. Radyofarmasötikler için kalite kontrol yöntemleri kılavuzu. Turk J Nucl Med 2003;12:137-148.
14. Decristoforo C, Zolle I, Rakiás F, Imre J, Jánoki G, Hesslewood SR. Quality control methods of 99mTc pharmaceuticals. In: Zolle I (eds). Technetium-99m Pharmaceuticals. Springer, Berlin, Heidelberg, 2007. https://doi.org/10.1007/978-3-540-33990-8_9
15. Wongkrajang P. Comparison of leukocyte differential count by beckman coulter unicel DxH800, Beckman Coulter LH780 and Sysmex XE-5000. Siriraj Medical Journal 2015;67.
16. Owunwanne A. The handbook of radiopharmaceuticals. Springer, 2012.
17. O'Malley JP, Ziessman HA. Nuclear medicine and molecular imaging: the requisites e-book. Elsevier Health Sciences, 2020.