Annals of Nuclear Medicine Vol. 10, No. 4, 419–423, 1996

Evaluation of Tc-99m(V) DMSA for imaging inflammatory lesions: An experimental study

Meral T. ERCAN,* Nedim C.M. GÜLALDI,* Işıl S. ÜNSAL,* Mehmet AYDIN,*
İrfan PEKSOY* and Zafer HASÇELİK**

*Departments of *Nuclear Medicine and **Physical Medicine and Rehabilitation, Faculty of Medicine, Hacettepe University, Ankara, Turkey*

The present study evaluated ^{99m}Tc(V) DMSA as an agent for the visualization of inflammatory lesions in comparison to ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG. All three radiopharmaceuticals were prepared with commercial kits. ^{99m}Tc(V) DMSA was prepared at neutral pH by the addition of first bicarbonate and then pertechnetate to the kit contents. The labeling efficiency was 99% as determined by ITLC. Abscesses were induced by i.m. injection of 50 μl turpentine into the right thighs of 36 Swiss albino mice. Six days later 3.7 MBq of each radiopharmaceutical was i.v. administered to 12 mice. The mice were sacrificed at 1, 3, 6 and 24 h later. Scintigrams were obtained with a gamma camera. The abscesses were better visualized on scintigrams with ^{99m}Tc(V) DMSA compared to ^{99m}Tc(III) DMSA, starting at 1 h. The animals were dissected and the organs were removed, weighed and the radioactivity determined with a gamma counter. The abscess to other tissue ratios were higher with ^{99m}Tc(V) DMSA than the other radiopharmaceuticals. The max. abscess/muscle ratios were 9.46 ± 3.20 (24 h), 4.19 ± 1.39 (6 h) and 5.98 ± 1.17 (24 h) and max. abscess/blood ratios were 6.22 ± 1.41, 4.09 ± 0.84 and 0.914 ± 0.351 all at 24 h for ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG, respectively.

Experimental arthritis was produced in 6 New Zealand white rabbits by intra-articular injection of ovalbumin. Four days later 37 MBq of ^{99m}Tc(V) DMSA and ^{99m}Tc-HIG were each i.v. administered to 3 rabbits. Scintigrams obtained at 1, 3, 6, and 24 h clearly demonstrated arthritic joints. ROI's over arthritic joints were compared to contralateral normal joints (A/C). The max. A/C ratios were 2.10 ± 0.31 (3 h) and 2.92 ± 0.99 (24 h) for ^{99m}Tc(V) DMSA and ^{99m}Tc-HIG, respectively.

Our results indicated the feasibility of imaging inflammatory lesions with ^{99m}Tc(V) DMSA.

Key words: ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA, ^{99m}Tc-HIG, inflammation, arthritis

INTRODUCTION

THE ACCUMULATION of ^{99m}Tc(V) DMSA (dimercaptosuccinic acid) by tumoral tissues has been well documented by both experimental[1–3] and clinical studies.[4–7] The chemical identity of the pentavalent state was determined by analytical methods[1,8] and its efficacy in imaging soft tissue tumors was compared with the commonly used renal agent ^{99m}Tc(III) DMSA both in mice[1] and humans.[9] The biodistribution in mice showed faster blood clearance and higher bone and muscle uptake with ^{99m}Tc(V) DMSA compared to ^{99m}Tc(III) DMSA.[1] In man tumors and metastases were well delineated with Tc in the pentavalent but not in the trivalent state,[9] although the ligand was the same. Tumor affinity of ^{99m}Tc(V) DMSA was attributed to the structural similarity of TcO_4^{-3} core to PO_4^{-3} ion.[4]

^{99m}Tc(V) DMSA is a nonspecific agent and as such it is expected to localize in inflammatory lesions like other small molecular weight coordination complexes of Tc

Received June 6, 1996, revision accepted September 19, 1996.

For reprint contact: Prof. Dr. Meral T. Ercan, Hacettepe Üniversitesi, Tıp Fakültesi, Nükleer Tıp Anabilim Dalı, 06100 Sıhhiye, Ankara, TURKEY.

This work was presented at the 42nd Annual Meeting of The Society of Nuclear Medicine, Minneapolis, MN, 11–15 June, 1995.

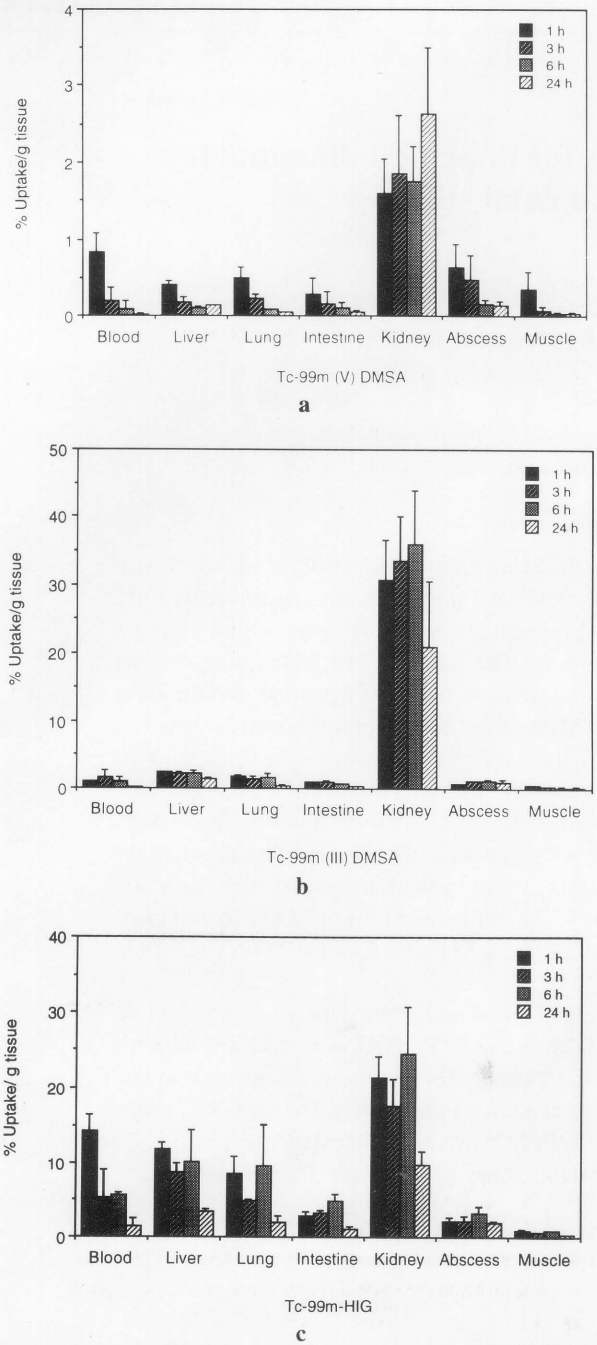


Fig. 1 Biodistribution of a) ^{99m}Tc(V) DMSA, b) ^{99m}Tc(III) DMSA and c) ^{99m}Tc-HIG in mice with turpentine-induced abscesses in right thighs.

mainly by a common mechanism of infiltration into the interstitial space due to increased capillary permeability.[10] Uptake of ^{99m}Tc(V) DMSA by inflammatory tissues has been reported by several groups,[4,5,7,11] but not fully investigated. It has the advantages of an ideal ^{99m}Tc label, simple preparation method from commercially available DMSA kits and being inexpensive. It is a candidate for wide usage as an agent for imaging inflammation in clinical application if the efficacy is demonstrated. In the present study the feasibility of scintigraphic imaging of experimental abscesses and arthritis was investigated in a comparative study with ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG (human immuno gamma globulin) in the hope of subsequent clinical application.

MATERIALS AND METHODS

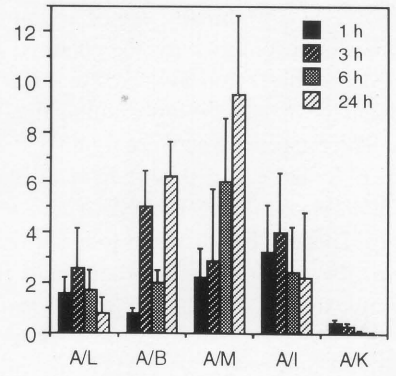
Radiopharmaceuticals

All three radiopharmaceuticals (RPs) were prepared with commercial kits. Kits for preparing ^{99m}Tc(III) DMSA were purchased from Sorin Biomedica S.p.a., Italy. The technetium generators and HIG kits were obtained from Mallincrodt Medical B.V., Holland. ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG were prepared by the addition of appropriate amounts of ^{99m}Tc-pertechnetate to the kit vials according to the instructions supplied by the manufacturers. ^{99m}Tc(V) DMSA was prepared according to the previously published procedure:[12,13] 0.12 m*l* sterile 7% solution of $NaHCO_3$ was added to the lypophylized kit material. When the solid material was dissolved completely 740 MBq ^{99m}Tc-pertechnetate in 1–3 m*l* was injected into the vial and the mixture was incubated at room temperature for 10 minutes. All three RPs were analyzed by impregnated-thin-layer-chromatography (ITLC) with ITLC-SG mini-strips and methyl ethyl keton or saline as solvents. The labeling efficiency was 99% for all three RPs.

Animal studies

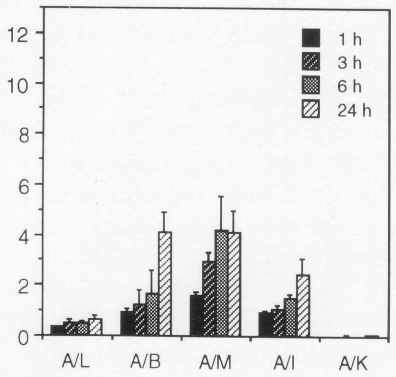
The animal studies were carried out in accordance with the British animal protection practice (UFAW Handbook, 2nd Edn). 50 μl turpentine was injected into the right thigh muscle of each of 36 Swiss albino mice. They were divided into 3 groups of 12 mice. Six days later the mice were injected through the tail vein with 3.7 MBq in 0.1 m*l* of ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA or ^{99m}Tc-HIG. They were sacrificed in groups of 3 at 1, 3, 6, and 24 h. Static images of the mice were obtained with a gamma camera (Toshiba GCA 601 E), fitted with a LEAP collimator. The mice were dissected. All the organs, the abscess, some muscle tissue from the contralateral leg, and blood and urine samples were obtained, weighed and counted in a gamma counter (Berthold, BF 5300, Germany) against a standard prepared from 1/100 dilution of the injected solution. % uptake/g tissues, and abscess/liver (A/L), blood (A/B), muscle (A/M), intestine (A/I) and kidney (A/K) ratios were calculated.

Experimental arthritis was produced in 6 New Zealand white rabbits (3.0–3.5 kg) by intra-articular injection of 1 m*l* ovalbumin (Sigma, U.S.A.) in 0.9% saline (20 mg/m*l*) emulsified with an equal volume of Freund's incomplete adjuvant as an antigen into the left front knee according to previous methods.[14,15] Four days later 3 rabbits were i.v. injected with 37 MBq ^{99m}Tc(V) DMSA and the other 3 with ^{99m}Tc-HIG. Scintigrams were obtained at 1, 3, 6 and 24 h. The regions of interest (ROIs) over arthritic (A) and



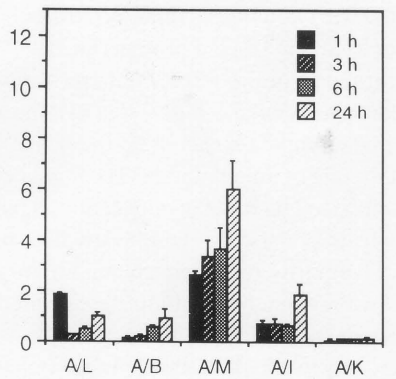
Tc-99m (V) DMSA

a



Tc-99m (III) DMSA

b



Tc-99m-HIG

c

Fig. 2 Abscess to other tissue concentration ratios for a) ^{99m}Tc(V) DMSA, b) ^{99m}Tc(III) DMSA and c) ^{99m}Tc-HIG obtained from biodistribution studies in mice with turpentine induced abscesses (A: abscess, L: liver, B: blood, M: muscle, I: intestine, K: kidney).

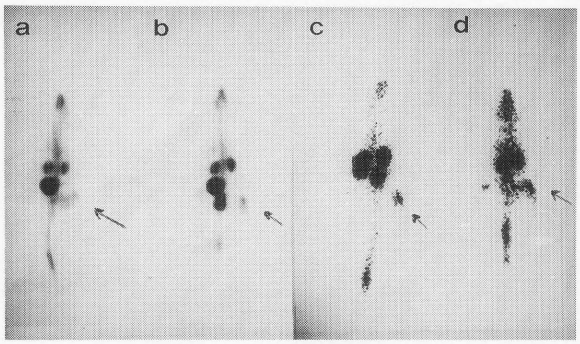
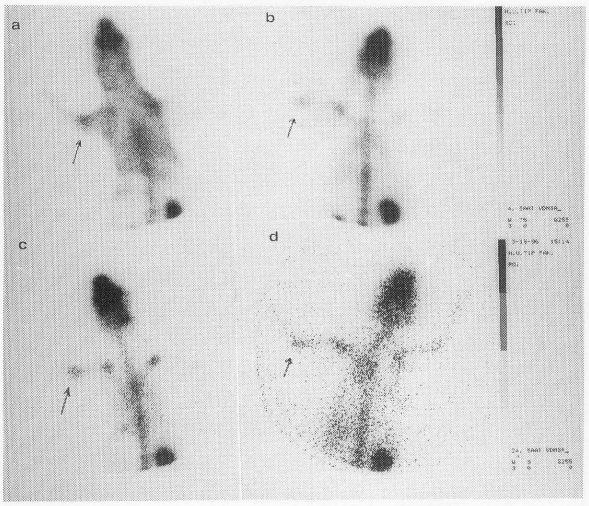
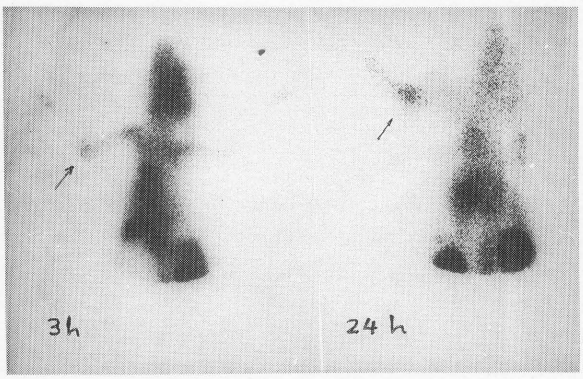


Fig. 3 Scintigrams obtained with ^{99m}Tc(V) DMSA in mice with turpentine-induced abscesses in right thighs at a) 1 h, b) 3 h, c) 6 h, and d) 24 h post-injection (arrows indicate abscesses).



A



B

Fig. 4 Scintigrams obtained with A) ^{99m}Tc(V) DMSA at a) 1 h, b) 3 h, c) 6 h, and d) 24 h post-administration and B) ^{99m}Tc-HIG at times indicated in rabbits with arthritic joints (arrows).

contralateral (C) normal knees were compared to obtain A/C ratios.

RESULTS

The biodistributions of ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG in mice with abscesses are shown in Fig. 1a–c. The maximum uptake was observed in the kidneys with all the RPs compared to other organs. The max. absolute uptakes as % injected dose/g tissue were 2.64 ± 0.87 (24 h), 36.1 ± 7.8 (6 h) and 24.5 ± 6.3 (6 h) for ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG, re-

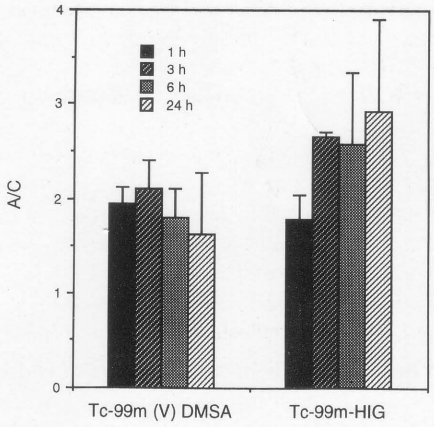


Fig. 5 Arthritic to contralateral tissue (A/C) ratios obtained from regions of interest on scintigrams in rabbits.

spectively. Most of the radioactivity administered was excreted by the kidneys in the case of ^{99m}Tc(V) DMSA compared to ^{99m}Tc(III) DMSA. The urinary excretion values not shown on the graphs were 116.1 ± 15.1, 120.4 ± 29.1 and 90.4%/g in the first hour for ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG, respectively.

The A/L, A/B, A/M, A/I and A/K ratios obtained in biodistribution studies are shown in Fig. 2a–c. Higher ratios were obtained with ^{99m}Tc(V) DMSA than with the other agents. The max. A/M ratio was 9.46 ± 3.20 at 24 h with ^{99m}Tc(V) DMSA compared to 5.98 ± 1.17 obtained with ^{99m}Tc-HIG. The 6 h ratio for ^{99m}Tc(V) DMSA (6.00 ± 2.52) was also higher than that for ^{99m}Tc-HIG (3.62 ± 0.86). The higher SD's of A/M obtained with ^{99m}Tc(V) DMSA may be due to individual variations in blood clearance and renal excretion. The abscesses were better visualized on scintigrams with ^{99m}Tc(V) DMSA starting at 1 h (Fig. 3) than with ^{99m}Tc(III) DMSA. The organs that were visualized were the kidneys and the urinary bladder.

Scintigrams of rabbits demonstrated arthritic joints with both ^{99m}Tc(V) DMSA and ^{99m}Tc-HIG (Fig. 4). There was less blood background with ^{99m}Tc(V) DMSA than with ^{99m}Tc-HIG during 1–6 h post-injection. The bone uptake more evident in ^{99m}Tc(V) DMSA images of the rabbits than in mice scintigrams is in line with earlier reported biodistribution studies.[1–3] For both RPs A/C ratios obtained by ROIs on scintigrams of rabbits are presented in Fig. 5. A/C ratios of ^{99m}Tc-HIG were a little superior after 3 h and kept increasing up to 24 h while ^{99m}Tc(V) DMSA showed a decline after 3 h, indicating a wash-out of radioactivity from the joints involved.

DISCUSSION

Our results demonstrated the accumulation of ^{99m}Tc(V) DMSA in experimental abscesses and arthritis. Rapid blood clearance and excretion predominantly via the kidneys may be responsible for the higher abscess/other tissue concentration ratios than with ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG. Due to the absence of abdominal (liver and intestines) radioactivity it has the potential of demonstrating lesions in this region compared to ^{99m}Tc-HIG. The only disadvantage may be the low absolute uptakes by the abscesses. The max. uptakes were 0.645 ± 0.268 (1 h), 1.17 ± 0.03 (3 h) and 3.20 ± 0.94 (6 h) % injected dose/g for ^{99m}Tc(V) DMSA, ^{99m}Tc(III) DMSA and ^{99m}Tc-HIG, respectively. This can be attributed to faster renal excretion as was also reported with other small molecular weight complexes of ^{99m}Tc that are mainly excreted via kidneys.[10,15,16] The abscess-to-other tissue concentration ratios obtained with ^{99m}Tc(V) DMSA are higher than those obtained with agents previously tested in our laboratory,[10,15,16] but the A/C values in rabbits are somewhat lower.[15,16] In two patients with active tuberculosis the quality of ^{99m}Tc(V) DMSA scintigrams was superior to those of ^{99m}Tc-citrate.[11] More comparative studies are necessary to prove the superiority of this new radiopharmaceutical. It is evident that ^{99m}Tc(III) DMSA is not suitable for this purpose because of its lower concentration ratios obtained compared to the other two agents. Furthermore, high renal cortical localization of ^{99m}Tc(III) DMSA results in an unacceptably high radiation dose to the kidneys, since an adult dose of 555–740 MBq is necessary in human application.

The main localization mechanism for all the three agents may be infiltration into the interstitial space due to increased capillary permeability. The increase in A/M ratios as time progressed may be a result of high protein binding of DMSA complexes reported earlier.[2] They also therefore behave like labeled proteins such as ^{99m}Tc-HIG and are entrapped in the interstitial space due to their assumed large molecular weight. It is also possible that ^{99m}Tc DMSA complexes or ^{99m}Tc itself are bound to proteins at the site of inflammation. The higher concentration ratios obtained with the pentavalent compared to the trivalent state of Tc in DMSA complexes also suggest that a structural similarity to the phosphate ion might play a role in its localization. More studies are needed to elucidate the localization mechanisms.

Commercially available nonspecific radiopharmaceuticals that are currently used for imaging inflammatory lesions are either macromolecules themselves such as ^{99m}Tc-HIG or assume a large molecular weight after i.v. administration such as ^{67}Ga-citrate from which ^{67}Ga dissociates and binds to transferrin in plasma.[17] They have prolonged blood clearance and high abdominal localization, necessitating delayed imaging. Small molecular weight complexes such as ^{99m}Tc-citrate,[15] ^{99m}Tc-DTPA,[10,18] ^{99m}Tc-glucoheptonate,[19] and ^{99m}Tc(V) DMSA may be better alternatives especially for lesions located in the abdominal region. They need to be further evaluated in comparative studies.

In conclusion, ^{99m}Tc(V) DMSA is preferred to ^{99m}Tc-HIG, because of the higher concentration ratios attained earlier, lower blood background, absence of radioactivity

in abdominal organs such as the liver and intestines and lower cost, but it should be compared to other renal agents in clinical application to find the best radiopharmaceutical for imaging inflammatory lesions in man.

ACKNOWLEDGMENT

This work was supported by the Turkish Scientific and Technical Research Council (TÜBİTAK) (Project No: SBAGD-AYD-47).

REFERENCES

1. Yokoyama A, Hata N, Horiuchi K, et al. The design of a pentavalent ^{99m}Tc-dimercaptosuccinate complex as a tumor imaging agent. *Int J Nucl Med Biol* 12: 273–279, 1985.
2. Chauhan UPS, Babbar A, Kashyap R, et al. Evaluation of a DMSA kit for instant preparation of ^{99m}Tc(V)-DMSA for tumour and metastasis scintigraphy. *Nucl Med Biol* 19: 825–830, 1992.
3. Watkinson JC, Allen SJ, Laws DE, et al. The pharmacokinetics and biodistribution of technetium-99m(V) dimercaptosuccinic acid in an animal model. *J Nucl Med* 32: 1235–1238, 1991.
4. Ohta H, Endo K, Fujita T, et al. Imaging of soft tissue tumors with Tc(V)-99m dimercaptosuccinic acid. A new tumor-seeking agent. *Clin Nucl Med* 9: 568–573, 1984.
5. Watkinson JC, Larazus CR, Mistry R, et al. Technetium-99m(V) dimercaptosuccinic acid uptake in patients with squamous carcinoma of the head and neck—Experience in imaging. *J Nucl Med* 30: 174–180, 1989.
6. Kashyap R, Babber A, Sahai I, et al. Tc-99m(V) DMSA imaging. A new approach to studying metastases from breast carcinoma. *Clin Nucl Med* 17: 119–122, 1992.
7. Kobayashi H, Sakahara H, Hosono M, et al. Soft tissue tumors: Diagnosis with Tc-99m(V) dimercaptosuccinic acid scintigraphy. *Radiology* 190: 277–280, 1994.
8. Blower PJ, Sing J, Clarke SEM. The chemical identity of pentavalent technetium-99m-dimercaptosuccinic acid. *J Nucl Med* 32: 845–849, 1991.
9. Ohta H, Ishii M, Yoshizumi M, et al. A comparison of the tumor-seeking agent Tc-99m(V) dimercaptosuccinic acid and the renal imaging agent Tc-99m dimercaptosuccinic acid in humans. *Clin Nucl Med* 10: 167–170, 1985.
10. Ercan MT, Ünlenen E. Accumulation of some small molecular weight complexes of ^{99m}Tc in experimental abscesses. *Nucl Med Biol* 21: 143–149, 1994.
11. Gülaldi NCM, Bayhan H, Ercan MT, et al. The visualization of pulmonary tuberculosis with Tc-99m(V) DMSA and Tc-99m citrate in comparison to Ga-67 citrate. *Clin Nucl Med* 20: 1012–1014, 1995.
12. Sampson C. Preparation of Tc-99m(V) DMSA. *Nucl Med Commun* 8: 184–185, 1987.
13. Babber A, Kashyap R, Chauhan UPS. A convenient method for the preparation of ^{99m}Tc-labelled pentavalent DMSA and its evaluation as a tumor imaging agent. *J Nucl Biol Med* 35: 100–104, 1991.
14. Consden R, Doble A, Glynn LE, et al. Production of a chronic arthritis with ovalbumin. Its retention in the rabbit knee joint. *Ann Rheum Dis* 30: 307–315, 1971.
15. Ercan MT, Aras T, Ünlenen Eş et al. ^{99m}Tc-Citrate versus ^{67}Ga-citrate for the scintigraphic visualization of inflammatory lesions. *Nucl Med Biol* 20: 881–887, 1993.
16. Ercan MT, Ünlenen E, Aktaç A. ^{99}Tc^{m}-glutathione for imaging inflammatory lesions. *Nucl Med Commun* 15: 533–539, 1994.
17. Tzen KY, Oster ZH, Wagner HN, et al. Role of iron-binding proteins and enhanced vascular permeability in the accumulation of gallium-67. *J Nucl Med* 21: 31–35, 1980.
18. Hosain F, Haddon MJ, Hosain H, et al. Radiopharmaceuticals for diagnosis and treatment of arthritis. *Nucl Med Biol* 17: 151–155, 1990.
19. Roizenblatt J, Buchpiguel CA, Menguetti JC, et al. Quantification of ocular inflammation with technetium-99m glucoheptonate. *Eur J Nucl Med* 18: 955–958, 1991.