# ACCUMULATION OF $^{99M}$TC LABELED RENAL AGENTS IN EXPERIMENTAL ABSCESSES

MERAL T. ERCAN*
NEDIM C. M. GÜLALDI*
JELALEDDIN SASANI*
ERKAN ÜNLENEN*
ISIL S. ÜNSAL*
ÜNSER ARIKAN**

*SUMMARY : The purpose of the present investigation was to evaluate the feasibility of imaging inflammatory lesions with some renal agents such as $^{99m}$Tc labeled gluconate (G), glucoheptonate (GH) and dimercaptosuccinic acid (DMSA) in comparison to $^{99m}$Tc-human immunogamma globulin (HIG).*

*The biodistributions of all the four radio-pharmaceuticals (RP's) were determined in mice with turpentine-induced abscesses at 1, 3, 6 and 24 hrs post-injection of 15 MBq in 0.1 ml solution of each RP. The % uptake/g tissues and abscess (A) over muscle (A/M), blood (A/B), liver (A/L), intestine (A/I) and kidney (A/K) concentration ratios were calculated. The scintigraphic images of all mice were obtained by a gamma camera.*

*The abscesses were well visualized with all the RP's. The excretion was mainly via the kidneys except for 99mTc-HIG which showed variable amounts of liver and kidney uptake. Similar A/M ratios were obtained, reaching a maximum at 6 hrs with $^{99m}$Tc- DMSA and 24 hrs with the others. The maximum values were 5.21±1.24, 3.60±0.52, 4.19±1.39 and 5.98±1.17 with $^{99m}$Tc-G, -GH, -DMSA and -HIG, respectively.*

*Our results indicated that $^{99m}$Tc renal agents can be used in imaging inflammation. They are preferred to $^{99m}$Tc-HIG, because of rapid blood clearance, excretion predominantly via the kidneys, no significant uptake by other organs, low cost and easy availability.*

*Key Words: $^{99m}$Tc-gluconate, -glucoheptonate, -DMSA, -HIG, turpentine-induced abscesses, inflammation.*

## INTRODUCTION

Scintigraphic visualization of abdominal abscesses offers a great challenge in nuclear medicine. $^{67}$Ga - Citrate introduced for imaging inflammatory lesions and widely used in clinics due to its high sensitivity is non-specific (1,2). In addition, its localization in the liver and intestines as a result of its biliary excretion prevents easy identification of inflammatory lesions in the abdominal region. Moreover, $^{67}$Ga has suboptimal physical characteristics, namely a long physical half-life, giving rise to a high radiation burden to patients and multiple gamma rays of which the energies are unsuitable for present-day cameras. Labeled proteins such as human serum albumin (HSA), immunogamma globulin (HIG) and antibodies have prolonged blood clearance, contributing significantly to background activity and liver uptake (3,4). Similar shortcomings can be expected with labeled leukocytes or granulocytes (5-7). Small molecular weight compounds with renal excretion can be better alternatives to proteins in imaging inflammation. There are isolated reports in recent scientific literature of the accumulation of $^{99m}$Tc-DTPA (8), -glucoheptonate (GH) (9), -citrate (10) and -glucose phosphate (GP) (11) in inflammatory processes. They have in

*From Department of Nuclear Medicine, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.
**From Department of Pathology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.

common fast renal clearance, no or minimal biliary excretion and no significant uptake by any other organs.

This study was undertaken to test some $^{99m}$Tc labeled renal agents such as gluconate (G), glucoheptonate and dimercapto-succinic acid in mice with turpentine-induced abscesses. The results were compared to those obtained with $^{99m}$Tc-HIG with the purpose of obtaining the best agent for imaging inflammation.

## MATERIALS AND METHODS

$^{99m}$Tc generator and DMSA kits were obtained from Amersham International, Amersham, U. K. Kits of gluconate were obtained from Izinta, Hungary. HIG kits were purchased from Mallinckrodt Medical B. V., Holland. They were labeled with $^{99m}$Tc according to the directions supplied by the manufacturers. $^{99m}$Tc-GH was prepared in-house by stannous chloride reduction method according to the previously published procedures (12). The labeling efficiency was determined at 15 min. after preparation of each agent with 'Impregnated Thin-Layer Chromatography' (ITLC) ready plates (ITLC-SG) obtained from Gelman Scientific Co., U.S.A. These plates were cut into 1x10 cm strips. Using the mini-chromatography technique and solvents such as physiological saline or acetone each agent was analyzed as described before (10).

### Animal studies

The animal studies were carried out in accordance with the British animal protection practice (13). Turpentine-induced abscesses were produced in 48 Swiss albino mice weighing 20-25 g by injection of 50 ml turpentine into the right thigh muscle. The biodistribution studies were carried out when the abscess age was 6 days (14). 12 mice were injected with 15 MBq $^{99m}$Tc-G in 0.1 ml through the tail vein. They were killed by decapitation in groups of 3 at 1, 3, 6, and 24 h. Static images of all mice were obtained by a gamma camera (Toshiba GCA 601E), using a LEAP collimator. ROI's over abscesses and contra lateral tissues were compared. The mice were dissected. The organs such as the liver, spleen, stomach, heart, lungs, intestines, pancreas, kidneys, the whole abscesses and some skeletal muscle from the contra lateral leg were removed. Blood and urine when available were also obtained. The organs and tissues were weighed and counted at the photo peak of $^{99m}$Tc (140 keV) in the gamma counter against a standard prepared from 1/100 dilution of the injected solution. The percentage uptake of each organ or tissue and % injected dose/g tissue were calculated. The means with standard deviations were computed. The abscess (A) uptake as % injected dose/g was compared to the uptake in muscle (M), blood (B), liver (L), intestines (I) and kidneys (K) in order to obtain tissue concentration ratios. The same procedure was followed with the other RP's: $^{99m}$Tc-GH, $^{99m}$Tc-DMSA and $^{99m}$Tc-HIG. The A/M, A/B, A/L, and A/K ratios for all the agents tested were plotted for comparison.

## RESULTS

All the RP's were prepared with high labeling efficiency (> 99) as determined by ITLC. They were stable up to 24 h of follow-up. Biodistributions of $^{99m}$Tc-G, -GH, -DMSA, and -HIG are presented in Tables 1, 2, 3, and 4, respectively. All the RP's were excreted by the kidneys as is evidenced by high renal uptakes and urinary radioactivity. The highest renal uptake per g tissue was observed in DMSA > G > HIG > GH.

There was no significant uptake in any other organs besides kidneys with $^{99m}$Tc-G, -GH and -DMSA with low blood background. On the other hand liver accumulation with high blood radioactivity levels was evident with $^{99m}$Tc-HIG.

The A/M, A/B, A/L, A/I, and A/K ratios obtained from biodistribution studies of all the RP's are presented in Figures 1a, 1b, 1c, 1d, 1e. According to A/M ratios all the RP's can be used for imaging abscesses. The maximum ratios were 5.21±1.24 (24 h), 3.60±0.52 (24 h), 4.19±1.39 (6 h) and 5.98±1.17 (24 h) for G, GH, DMSA and HIG, respectively, indicating the superiority of HIG. However, with $^{99m}$Tc complexes, it is imported that the imaging is performed in a few hours post-injection because the image

Table 1: Biodistribution of $^{99m}$Tc-G in mice with turpentine-induced abscesses.

| % Uptake /g tissue (mean ± S.D.) | | | | |
|---|---|---|---|---|
| Organ | 1h | 3h | 6h | 24h |
| Blood | 1.19±0.45 | 0.318±0.225 | 0.243±0.103 | 0.102±0.074 |
| Liver | 2.02±0.102 | 1.19±0.22 | 0.988±0.009 | 0.398±0.043 |
| Spleen | 0.330±0.158 | 0.212±0.011 | 0.167±0.010 | 0.118±0.010 |
| Stomach | 0.239±0.049 | 0.242±0.127 | 0.490±0.136 | 0.259±0.283 |
| Heart | 0.497±0.264 | 0.248±0.032 | 0.199±0.101 | 0.657±0.0404 |
| Lungs | 1.38±0.63 | 0.606±0.043 | 0.714±0.087 | 0.216±0.034 |
| Intestines | 1.71±0.59 | 1.16±0.25 | 1.05±0.29 | 0.157±0.005 |
| Pancreas | 0.541±0.215 | 0.253±0.024 | 0.245±0.032 | 0.0942±0.0017 |
| Kidneys | 25.43±1.93 | 15.84±2.35 | 11.54±0.36 | 5.24±1.72 |
| Abscess | 0.733±0.28 | 0.494±0.025 | 0.378±0.033 | 0.245±0.016 |
| Muscle | 0.313±0.065 | 0.164±0.023 | 0.138±0.015 | 0.0496±0.0011 |

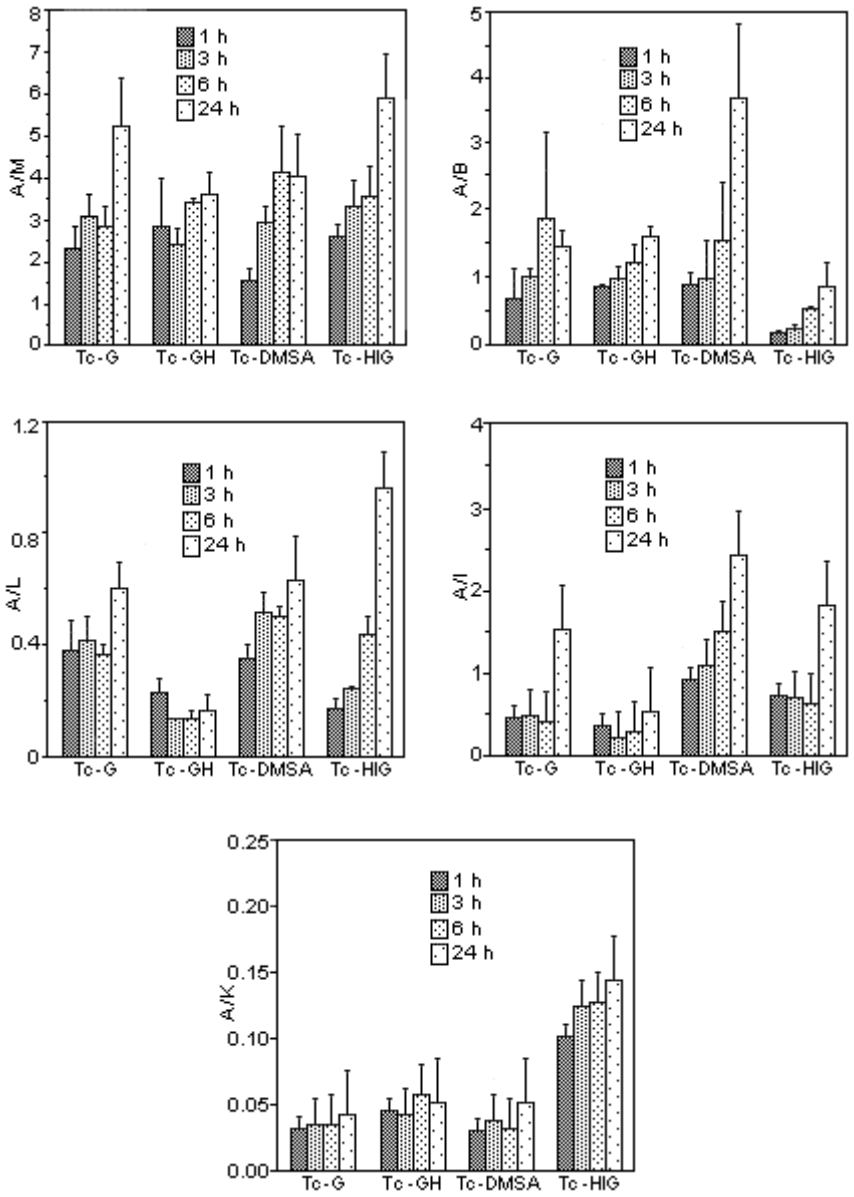



Figure 1: Abscess/muscle (A/M), abscess/blood (A/B), abscess/liver (A/L), abscess/intestine (A/I) and abscess / kidney (A/K) concentration ratios obtained from biodistribution studies in mice with turpentine-induced abscessas (Tc:$^{99m}$Tc).

quality deteriorates with decreasing radioactivity due to physical decay. If the A/M ratios obtained within 6 h post-injection are compared $^{99m}$Tc-MA is better than $^{99m}$Tc-HIG. When A/B ratios are compared, all the 3 renal RP's are better than $^{99m}$Tc-HIG. A/L ratios of $^{99m}$Tc-DMSA and $^{99m}$Tc-G are also better than $^{99m}$Tc-HIG. Only $^{99m}$Tc-DMSA has better A/I ratios than $^{99m}$Tc-HIG. The A/K ratios of all the renal agents are lower than $^{99m}$Tc-HIG due to their renal excretion.

Scintigrams of all mice indicated the localization of all the RP's tested in abscesses. However, with $^{99m}$Tc-HIG there was high blood pool radioactivity in contrast to low blood background with the renal agents. The only organs visualized were the kidneys and the urinary bladder with the renal agents without any significant accumulation in the liver (Figures 2a, 2b, 2c). Figure 3 demonstrates an 8 mm section of a turpentine-induced abscess, confirming the abscess formation.

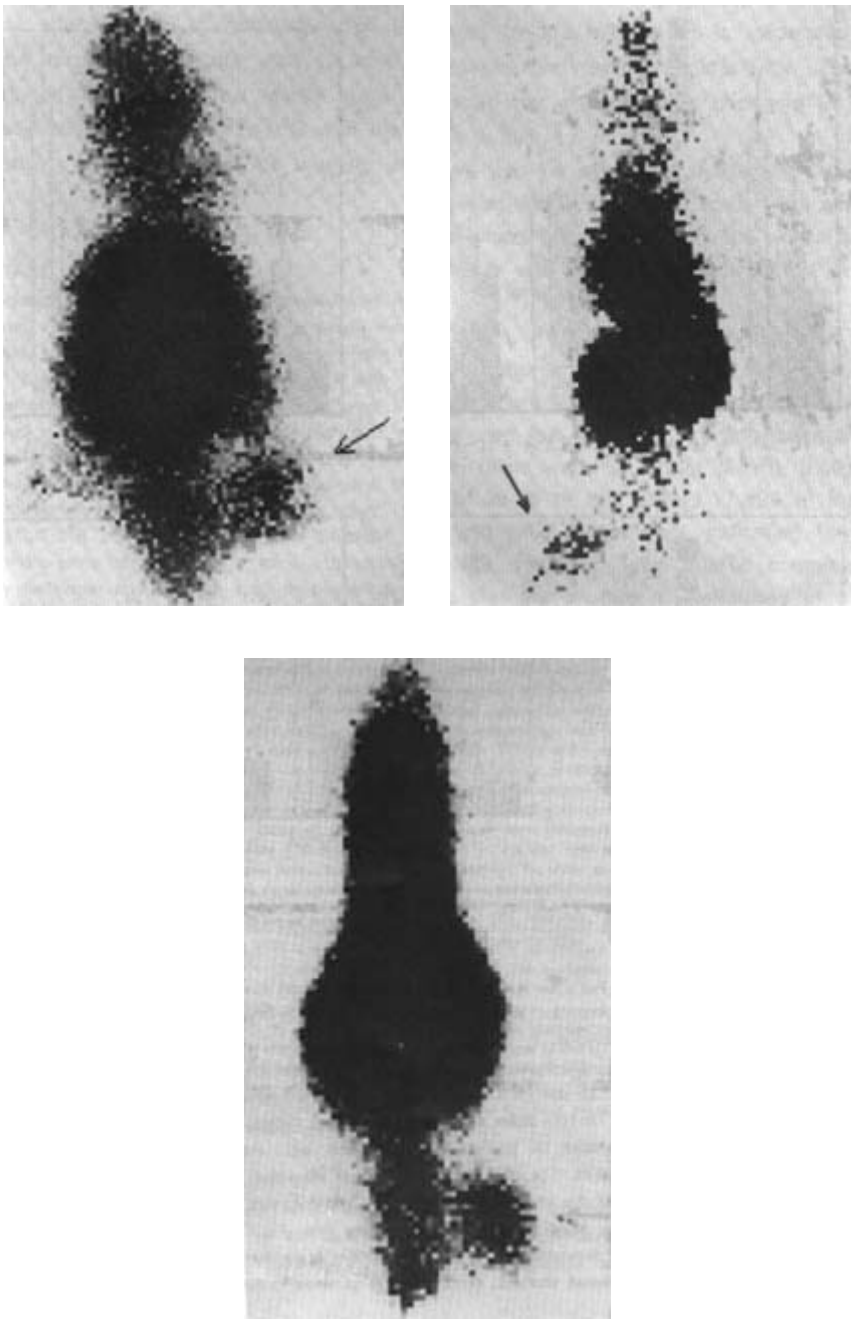


Figure 2: Scintigrams obtained in mice with turpentine-induced abscesses at 3 h post-injection of $^{99m}$Tc-G, $^{99m}$Tc-DMSA and $^{99m}$Tc-HIG.

DISCUSSION

In this investigation three renal RP's were compared to $^{99m}$Tc-HIG for accumulation in experimental abscesses. $^{99m}$Tc-HIG was proposed especially for this purpose and has been in clinical use since its introduction (15) with highly expensive commercial kits. We have demonstrated that it is possible to image inflammatory lesions with simple complexes of $^{99m}$Tc with fast blood clearance and renal excretion at a very low cost. Although $^{99m}$Tc-HIG was considered specific when proposed, later it was found out that it was non-specific like the other agents such as 67Ga citrate and proteins (3,16). The superiority of renal agents is

Table 2: Biodistribution of $^{99m}$Tc-GH in mice with turpentine-induced abscesses.

| % Uptake /g tissue (mean ± S.D.) | | | | |
|---|---|---|---|---|
| Organ | 1h | 3h | 6h | 24h |
| Blood | 0.680±0.057 | 0.222±0.165 | 0.242±0.084 | 0.0642±0.0469 |
| Liver | 2.201±0.103 | 2.344±0.154 | 2.076±0.234 | 1.076±0.152 |
| Spleen | 0.294±0.009 | 0.280±0.032 | 0.229±0.043 | 0.135±0.017 |
| Stomach | 0.351±0.125 | 1.052±0.675 | 0.300±0.092 | 0.0925±0.0179 |
| Heart | 0.275±0.038 | 0.225±0.089 | 0.137±0.017 | 0.0779±0.0074 |
| Lungs | 0.525±0.024 | 0.401±0.050 | 0.336±0.055 | 0.176±0.023 |
| Intestines | 1.414±0.109 | 1.864±0.500 | 1.202±0.241 | 0.354±0.075 |
| Pancreas | 0.523±0.064 | 0.466±0.025 | 0.470±0.051 | 0.236±0.014 |
| Kidneys | 11.81±1.25 | 8.47±0.54 | 4.86±1.02 | 2.61±0.46 |
| Abscess | 0.513±0.057 | 0.340±0.017 | 0.295±0.072 | 0.178±0.033 |
| Muscle | 0.220±0.098 | 0.145±0.015 | 0.0763±0.0184 | 0.0492±0.0058 |

due to low uptake by other organs or tissues, and no significant biliary excretion. The only other organ visualized besides kidneys was the urinary bladder which can be voided in man. Thus, it is possible to visualize the abdominal abscesses. Although the very high A/M and A/B ratios obtained with specific 111 In-leukocytes introduced by McAfee *et. al.* (4) were not attained by any of the RP's tested in this study in view of the disadvantages of cell harvesting and vitro labeling of leukocytes requiring time-consuming steps by trained personnel (17), $^{99m}$Tc renal agents offer an easy method of localizing inflammatory lesions. The kits of $^{99m}$Tc renal agents are inexpensive compared to HIG kits. They can also be prepared in-house by simple and rapid procedures. Among the three agents tested G and GH should be preferred to DMSA, because of their faster renal excretion. $^{99m}$Tc-DMSA accumulates in the renal cortex, thus, delivering a higher radiation dose to this organ. Since a 555 MBq or more radioactivity dose is considered for imaging inflammatory foci, the biological half-life of the RP should be short.

We believe that increased capillary permeability is the main underlying mechanism for the localization of all the RP's in experimental abscesses (3). The renal agents that are tested in the present investigation have smaller molecular weight than proteins. It is easier for small molecular weight compounds to leak out from the injured capillaries (8,18). The increase in concentration ratios at 24 h post-injection suggests that an additional mechanism might be in operation with these complexes of $^{99m}$Tc. This additional mechanism might be specific or nonspecific binding to proteins at the sites of inflammation so that back diffusion into blood is hindered.

In conclusion, the experimental studies indicated the feasibility of imaging inflammatory lesions with some renal

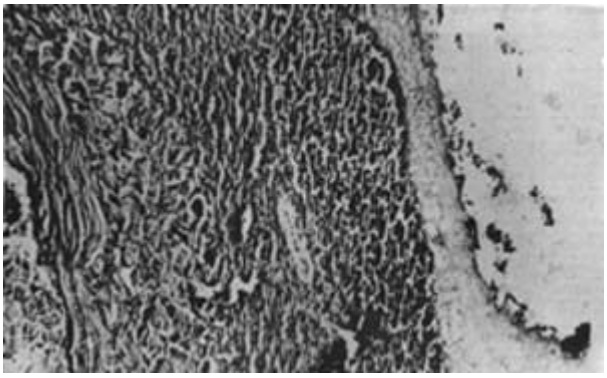
Table 3: Biodistribution of $^{99m}$Tc-DMSA in mice with turpentine-induced abscesses.

| % Uptake /g tissue (mean ± S.D.) | | | | |
|---|---|---|---|---|
| Organ | 1h | 3h | 6h | 24h |
| Blood | 0.955±0.121 | 1.56±1.12 | 1.04±0.68 | 0.257±0.043 |
| Liver | 2.38±0.12 | 2.22±0.28 | 2.13±0.42 | 1.44±0.19 |
| Spleen | 0.866±0.125 | 0.851±0.147 | 0.817±0.091 | 0.713±0.228 |
| Stomach | 0.325±0.060 | 0.318±0.050 | 0.279±0.103 | 0.138±0.044 |
| Heart | 1.17±0.14 | 0.871±0.0308 | 0.703±0.244 | 0.360±0.062 |
| Lungs | 1.79±0.14 | 1.50±0.40 | 1.71±0.51 | 0.489±0.0149 |
| Intestines | 0.933±0.067 | 1.08±0.11 | 0.7301±0.105 | 0.378±0.047 |
| Pancreas | 0.949±0.113 | 0.618±0.091 | 0.584±0.160 | 0.337±0.134 |
| Kidneys | 30.8±5.8 | 33.6±6.4 | 36.1±7.8 | 21.0±9.5 |
| Abscess | 0.867±0.030 | 1.17±0.03 | 1.13±0.26 | 0.969±0.359 |
| Muscle | 0.565±0.061 | 0.400±0.036 | 0.284±0.047 | 0.240±0.071 |
| Urine | 120.4±29.1 | - | 4.00±1.22 | 2.05±0.50 |

Table 4: Biodistribution of $^{99m}$Tc-HIG in mice with turpentine-induced abscesses (*only one urine sample).

| % Uptake /g tissue (mean ± S.D.) | | | | |
|---|---|---|---|---|
| Organ | 1h | 3h | 6h | 24h |
| Blood | 14.26±2.06 | 5.29±3.74 | 5.68±0.26 | 1.42±1.09 |
| Liver | 11.75±1.06 | 8.81±1.17 | 10.24±4.11 | 3.45±0.43 |
| Spleen | 3.57±0.63 | 2.57±0.22 | 3.34±1.35 | 1.11±0.12 |
| Stomach | 2.51±1.05 | 3.34±0.43 | 4.13±0.64 | 0.451±0.181 |
| Heart | 3.80±0.80 | 2.49±0.43 | 3.10±1.68 | 0.841±0.104 |
| Lungs | 8.62±2.23 | 4.88±0.17 | 9.57±5.57 | 1.99±1.01 |
| Intestines | 2.96±0.58 | 3.22±0.43 | 4.94±0.85 | 1.14±0.30 |
| Pancreas | 3.13±0.67 | 2.26±0.05 | 2.51±0.75 | 0.729±0.110 |
| Kidneys | 21.42±2.77 | 17.60±3.60 | 24.52±6.27 | 9.77±1.78 |
| Abscess | 2.21±0.46 | 2.26±0.68 | 3.20±0.94 | 2.02±0.19 |
| Muscle | 0.836±0.190 | 0.661±0.100 | 0.878±0.067 | 0.349±0.061 |
| Urine | 90.4** | 81.8** | - | - |

Figure 3: An 8 μm section of turpentine-induced abscess, showing from right to left: abscess cavity, fibrin formation and migration of leukocytes into interstitial space (x115).



agents. They are preferred to $^{99m}$Tc-HIG, due to early accumulation, rapid blood clearance by renal excretion, no significant uptake by other organs, low cost and easy availability.

REFERENCES

*1. Lavender JP, Loew J, Barker JR, Burn IJ and Chaudhri MA : Gallium-67 citrate scanning in neoplastic and inflammatory esions. Br J Radiol, 44:361-366, 1971.*

*2. Staak EV and McCartney WH : Role of gallium 67 in inflammatory disease. Semin Nucl Med, 8:219-234, 1978.*

*3. Thakur ML, Defulvio J, Park CH, Damjanov A, Yaghsezian H, Jungkind D, Epstein A and McAfee JG : Technetium-99m labeled proteins for imaging inflammatory foci. Nucl Med Biol, 18:605-612, 1991.*

*4. McAfee JG, Gagne G, Subramanian F and Schneider RF : The localization of indium-111-leukoctes, gallium-67-polyclonal IgG and other radioactive agents in acut focal inflammatory lesions. J Nucl Med, 32:2126-2131, 1991.*

*5. Lind P, Langsteger W, Költringer P, Dimai HP, Passe R and Eber O : Immunoscintigraphy of inflammatory processes with a technetium-99m labeled monoclonal antigranulocyte antibody (Mab BW 250/183). J Nucl Med, 31:417-423, 1990.*

*6. Vorne M, Soini J, Lantto T and Paakinen S : Technetium-99m-HMPAO-labelled leukocytes in detection of inflammatory lesions: Comparison with gallium-67 citrate imaging. J Nucl Med. 30:1322-1326, 1989.*

*7. Hotze A, Kropp J, Kozak, et. al. : $^{99m}$Tc-HMPAO leukocytes imaging: First clinical results and possible problems in inflammatory abdominal diseases. Nuc Med, 27:115-116, 1988.*

*8. Hosain F, Haddon MJ, Hosain H, Drost JK and Spencer RP : Radio pharmaceuticals for diagnosis and treatment of arthritis. Nucl Med Biol, 17:151-155, 1990.*

*9. Roizenblatt J, Buchpiguel CA, Menguetti JC, Caldeira JAF and Camargo EE : Quantification of ocular inflammation with technetium-99m-glucoheptonate. Eur J Nucl Med, 18:955-958, 1991.*

*10. Ercan MT, Aras T, Ünlenen E, Ünlü M, Ünsal IS and Hasçelik Z : $^{99m}$Tc-citrate versus 67Ga-citrate for the scintigraphic visualization of inflammatory lesions. Nucl Med Biol, 20:881-887, 1993.*

*11. Caner BE, Ercan MT, Bekdik CF, et. al. : Visualization of bone pathologies and lung cancer with $^{99m}$Tc-glucose phosphate: A comparative study. Nucl Med, 30:132-136, 1991.*

*12. Waxman AD, Tanacescu D, Siemsen JK and Wolfstein RS : Technetium-99m-glucoheptonate as a brain scanning agent. Critical comparison with pertechnetate. J Nucl Med, 16:580-581, 1975.*

*13. Hume CW : The legal protection of laboratory animals. In the UFAW Handbook on the Care and Management of Laboratory Animals (Ed by AN Worden and W Lane-Petter) Courier, pp 1-14, London, 1959.*

*14. Ercan MT, Aras T and Ünsal IS : Evaluation of $^{99m}$Tc-erytromycin and $^{99m}$Tc-streptomycin sulphate for the visualization of inflammatory lesions. Nucl Med Biol, 19:803-806, 1992.*

*15. Buscombe JR, Lui D, Ensing G, et. al. : Human immunoglobulins (HIG)- first results of a new agent for the localization of infections and inflammation. Eur J Nucl Med, 16:649-655, 1990.*

*16. Rusckowski M, Fritz B and Hnatowitch DJ : Localization of infection using streptavidin and biotin: An alternative to nonspecific conjugated polyclonal immunoglobulin. J Nucl Med, 33:1810-1815, 1992.*

*17. Mc Afee JG, Subramanian F and Gagne G : Technique of leukocyte harvesting and labeling; problems and perspectives. Sem Nucl Med, 14:83-104, 1984.*

*18. Ercan MT, Aras T, Ünsal IS, Arikan Ü, Ünlenen E and Hasçelik Z : Technetium-99m citrate for imaging Inflammation: An experimental study. J Islam Acad Sci, 5:180-188, 1992.*

Correspondence:
Meral T. Ercan
Hacettepe Üniversitesi,
Tip Fakültesi,
Nükleer Tip Bölümü,
06100 Sihhiye, Ankara,
TÜRKIYE.