TECHNETIUM-99M CITRATE FOR IMAGING INFLAMMATION: *AN EXPERIMENTAL STUDY*

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SUMMARY: Citric acid was labeled with ^{99m}Tc by the Sn^{++} reduction method with an efficiency of >98%. The biodistribution of ^{99m}Tc-citrate was determined in mice with abscesses in their right thigh muscles and compared to ^{67}Ga-citrate. The maximum abscesses/muscle ratios for ^{99m}Tc-citrate were 4.00±1.39 and 4.47±1.76 at 1 h for the turpentine- and Staphylococcus-induced abscesses, respectively. Corresponding values for ^{67}Ga-citrate were 4.76±2.04 and 3.97±1.99 at 4 h. Arthritis was induced in 10 rabbits with intra-articular injection of ovalbumin. Scintigraphic images obtained as a function of age up to 21 days showed increased activity involving the synovium. The maximum arthritic/contralateral knee ratio was 3.56±2.42 at 21 days. The blood clearance curve of ^{99m}Tc-citrate in rabbits was biexponential with a fast ($T_{1/2}$=51 min) and a slow ($T_{1/2}$=22 h) components. Analysis of urine samples indicated that most of the excreted radioactivity was due to intact ^{99m}Tc-citrate (>95%), as determined by ITLC and electrophoresis. Our results demonstrated that ^{99m}Tc-citrate is a potential radio pharmaceutical for the visualization of inflammatory lesions.

Key Words: Inflammation, technetium-^{99m} citrate, synovial scintigraphy.

INTRODUCTION

Many radiopharmaceuticals have been introduced in nuclear medicine for the scintigraphic visualization of focal inflammatory lesions (1,3,4,8,10,14,20,21,23,-24,27), including the more commonly used ^{67}Ga-citrate (8), ^{111}In (23) or ^{99m}Tc (10, 26) labeled leukocytes, ^{99m}Tc labeled granulocyte-specific monoclonal antibodies (Mab BW 250/183) for the in vivo labeling of leukocytes (9), ^{111}In (20) or ^{99m}Tc (1) labeled polyclonal human immunoglobulin (HIG). However, there is not a single ideal radio pharmaceutical that has found wide clinical application. The advantages and disadvantages of the proposed agents are fully discussed in current literature (12,13,16,17,25). In view of the very high target-to-nontarget ratios (13), sensitivity and specificity (7) obtained with labeled leukocytes, they are at present the agents of choice, despite the time-consuming steps involved in cell harvesting and labeling (11). In addition, the high cost of ^{111}In or HM-PAO kits used for ^{99m}Tc labeling has to be considered. Consequently, the search for better agents preferably labeled with ^{99m}Tc in a simple and rapid procedure for intravenous administration will continue until favorable characteristics of labeled leukocytes are attained.

The main problem with ^{67}Ga-citrate and labeled proteins has been the high levels of blood radioactivity which renders the differentiation of lesions against blood background difficult. An additional problem is excretion via biliary tract as in ^{67}Ga-citrate resulting in liver and intestinal accumulation of radioactivity, necessitating delayed studies for abdominal abscesses. Therefore, an ideal radio

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pharmaceutical for inflammatory lesions should have fast renal clearance without any liver uptake and biliary excretion in addition to high target-to-nontarget ratios. According to recent findings infiltration into interstitial space due to increased capillary permeability seems to be the main mechanism in abscess localization. Thakur *et al.* (24) obtained similar concentration ratios in experimental abscess when they compared ^{67}Ga-citrate, ^{99m}Tc or ^{125}I labeled proteins, including human serum albumin (HSA) and immunogamma globulin (IgG). Water soluble polar complexes of ^{99m}Tc with a small molecular weight and low plasma protein binding should be as good as or maybe better than the presently used radio pharmaceuticals for the visualization of inflammatory lesions, since they can penetrate the injured capillaries better and the remaining radioactivity easily excreted by kidneys. In this study ^{99m}Tc-citrate was chosen as a representative complex to test this hypothesis and evaluated in experimental models of inflammation in comparison to ^{67}Ga-citrate. It is one of the first ^{99m}Tc complexes introduced in nuclear medicine, well characterized and used as a renal agent (15).

MATERIALS AND METHODS

Radiopharmaceutical

The following procedure was used for labeling: 10 mg citric acid (Brothers Chem. Co., U.S.A.) was dissolved in 2 ml water in a glass vial. The pH was adjusted to 5 with 0.5 N NaOH. 0.2 ml $SnCl_2$ $2H_2O$ aq. solution (1 mg/ml) was added and mixed well. The mixture was passed through 0.22 μm Millipore membrane filter into a sterile vial. 1-2 ml generator (Amersham International, Amersham, U.K.) eluate containing 370-555 MBq of ^{99m}Tc as pertechnetate was added and left to react at room temperature (R.T.) for 10 min.

The labeling efficiency was determined by Impregnated-Thin-Layer-Chromatography, using ready plates of ITLC-SG (Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.) 5-15 min after ^{99m}Tc-citrate preparation. The sheets were cut into 1.2x10 cm strips. 10 μl samples were applied at a point 1 cm from one end. The solvents were allowed to reach 8 cm from the origin. The strips were cut in halves and the radioactivity in each segment was determined in a gamma well-type counter (Model: MF 5300, Berthold, F.R.G.). Pertechnetate moved with the solvent front and hydrolyzed-reduced (H-R) ^{99m}Tc remained at the point of application in both solvents. ^{99m}Tc-citrate remained at the origin in acetone, but moved with the solvent front in saline. The labeling efficiency was determined by subtracting the sum of the amounts that migrated in acetone and that remained at the origin in saline from 100%. The stability of ^{99m}Tc-citrate was tested at 0.5, 1, 3, and 24 h after storage at R.T. by using the same method.

The electrophoretic motility of ^{99m}Tc-citrate towards anode was determined in 3 buffer systems: 1) veronal (0.06 M, pH=5.5). Whatman 3 mm paper (2x60 cm) was used as the stationary phase. 10 μl of the prepared mixture was applied at a point 6 cm from one end. The electrophoresis was run for 2 h at 500 V using a H. V. electrophoresis apparatus (Pherograph 60, Hormuth-Vetter, F.R.G.).

Ten μl samples (10) of ^{99m}Tc-citrate were mixed with 2 ml saline and 2 ml n-butanol in glass tubes. They where shaken vigorously and rotated for 30 min on a rotator (Byk-Mallinckrodt). They were left at R.T. for 2 h for the phases to settle. 0.5 ml samples were taken from both the organic and aqueous phases and counted in the gamma counter. The percentage of radioactivity extracted into the organic phase was calculated.

Animal Studies

The animal studies were carried out in accordance with the British animal protection laws (UFAW Handbook, 2nd Edition). Turpentine-induced abscesses were produced in mice according to the method of Thakur *et al.* (24). 40 Swiss albino mice weighing 20-25 g were injected with 50 μl turpentine into the right thigh muscle. The biodistribution studies were carried out when the abscess age was 6 days instead of 2 days proposed by Thakur *et al.*, because it was easier to dissect and isolate the abscesses intact. 18 mice were injected with 3.7 MBq ^{99m}Tc-citrate in 0.2 ml through the tail vein. They were sacrificed 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 60 1E), using a LEAP collimator. Regions of interest (ROI's) were drawn over the abscess and contra lateral tissues and were compared. The mice were dissected. The organs such as liver, spleen, stomach heart, lungs, intestines, pancreas, kidneys, the whole abscess and some skeletal muscle from the contra lateral leg were removed. Some 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/l00 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 (SD) were computed. Six of the mice were injected with 3.7 MBq ^{67}Ga-citrate (Amersham International plc, Amersham, U.K.) in 0.1 ml and the above procedure was followed except that the animals were sacrificed in groups of 3 at 4 and 24 h post-injection, the scintigrams were taken, using a medium energy collimator and the tissues were counted with a wide window (200 keV) to include the two photo peaks (184 and 296 keV) of ^{67}Ga.

Bacterial abscesses were induced by the injection of Staphylococcus aureus (ATCC 25923). $5x10^9$ bacteria in 0.1 ml was injected into the right thigh muscle in 20 mice. After 6 days biodistribution studies with ^{99m}Tc-citrate and ^{67}Ga-citrate were repeated following the same procedure.

In order to find the effect of abscess age on the abscess/contra lateral tissue ratios of ^{99m}Tc-citrate, 12 mice with turpentine-induced abscesses were I.V. injected with 18.5 MBq ^{99m}Tc-citrate on 2, 6, 10, and 20 days following turpentine injection and were sacrificed 3 h later. The scintigrams were obtained and the ROI's over abscess and contra lateral tissues were compared.

Experimental arthritis was produced in 10 New Zealand White rabbits (2.5-3.0 kg) by intra-articular injection of 1 ml ovalbumin (Sigma, U. S. A.) in 0.9% saline (20 mg/ml) emulsified with an equal volume of Freund's incomplete adjuvant as an antigen into the right front knee according to previous methods (2, 18). The contra lateral knees were used as control joints. The animals were kept under supervision up to 21 days. On 4, 7, 13, and 21 days following the induction of arthritis scintigrams were obtained 3 h after the injection of 37 MBq ^{99m}Tc-citrate in 1 ml through the ear vein of 5 rabbits. ROI's over the arthritic and contra lateral knee joints were drawn and the counts were compared. 5 rabbits were I.V. injected with 18.5 MBq ^{67}Ga-citrate. Scintigrams were obtained at 4 and 24 h. Again the ROI's over arthritic and contra lateral normal knees were compared.

Blood clearance of ^{99m}Tc-citrate was studied in 5 normal rabbits. 10 MBq ^{99m}Tc-citrate in 1 ml was injected through the ear vein. Blood samples (1-2 ml) were obtained from a vein of the other ear at 5 min, 30 min, 1, 3, 6, and 24 h. They were counted in the gamma counter against a standard prepared from 1/100 dilution of the injected solution. The means of % injected dose/ml were plotted as a function of time.

In Vitro Studies

Plasma and urine samples obtained from mice at 1 and 3 h were analyzed by ITLC, using ITLC-SG mini-strips and solvents acetone or saline, and by electrophoresis (500 V, 2 h) with phosphate buffer (0.05 M, pH=7.5).

Freshly drawn human blood was used in the following experiments. 4 ml samples (5) were mixed with 3.7 MBq ^{99m}Tc-citrate and incubated at 37°C for 2 h. They were centrifuged for 15 min at 4000 rpm. Both the separated plasma and the red blood cells (RBC's) were counted in the gamma counter. The percentage ^{99m}Tc-citrate bound to RBC's were calculated. To check the stability of the erythrocyte binding of ^{99m}Tc-citrate, the labeled cells were repeatedly washed with physiological saline. 4 ml saline was added to the RBC's, mixed well, incubated at 37°C for 10 min and centrifuged for 10 min at 4000 rpm. The wash solution was removed and counted together with the RBC's. This procedure was repeated six times.

In vitro protein binding of ^{99m}Tc-citrate was determined in plasma samples by protein precipitation with trichloroacetic acid (TCA). To 0.5 ml plasma containing a few kBq ^{99m}Tc-citrate 5 ml of a 5% TCA solution were added. 10 min later the mixture was centrifuged for 10 min at 4000 rpm. The supernatant was decanted into another tube. 5 ml of TCA solution were added to the precipitate and the mixture was again centrifuged. The washing was repeated 5 times. Both the precipitate and the wash solutions were counted. The fraction of radioactivity precipitated with the proteins was calculated.

Table 1: Stability of ^{99m}Tc-citrate at room temperature.*

Elapsed time (h)	No. of observations	%		
		$^{99m}TcO4^{-+}$	H-R ^{99m} Tc$^{\neq}$	^{99m} Tc-citrate§
0.1-0.2	10	2.23±1.47	0.27±0.24	97.49±1.61
0.5	15	0.73±0.65	0.17±0.19	99.13±0.64
1	5	0.60±0.30	0.52±0.03	98.88±0.31
3	5	1.54±0.51	0.29±0.19	98.17±0.63
24	5	1.69±1.28	0.16±0.12	98.13±1.36

* All figures are mean±SD.
+% migration in methyl ethyl ketone (a)
$^{\neq}$% remaining at origin in saline (b)
§100-(a+b)

Pathology

Two mice with turpentine- and two mice with Staphylococcus-induced abscesses were killed 6 days after abscess induction and the abscesses were removed. They were fixed in buffered formaldehyde (10%) and embedded in paraffin blocks. 8 μm sections were taken. They were stained with haemotoxylin and eosin. Two rabbits were killed with an overdose of Nembutal at 21 days following the induction of arthritis. The right front legs were removed and fixed in formaldehyde. Bone was decalcified by 5% formic acid and was embedded in paraffin blocks. 8 μm sections were taken and stained as before.

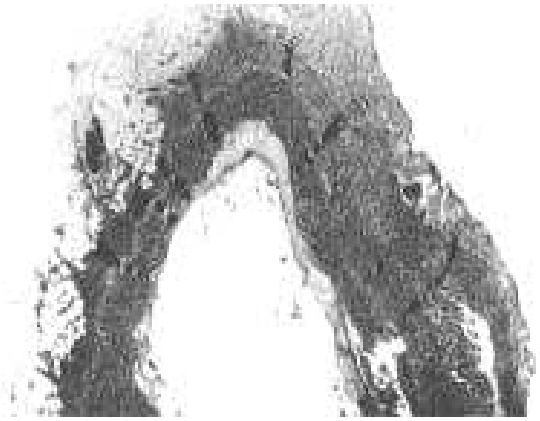
Table 2: Some properties of ^{99m}Tc-citrate.

Electrophoretic motility	0.0 with streaking towards anode
Extraction into n-butanol	0.343 ± 0.107 %
Stability in plasma	97.8 ± 0.71 %
Binding to serum proteins	18.06 ± 0.45 %
Erythrocyte binding	10.02 ± 0.82 %
Retention by RBC's after 6 washings	1.12 ± 0.15 %

RESULTS

Citric acid was labeled with ^{99m}Tc with a very high efficiency (>98%), as determined by ITLC and electrophoresis. The radiopharmaceutical was stable up to 24 h of testing (Table 1). On electrophoresis ^{99m}Tc-citrate remained at the point of application with much streaking. The inability to show any motility indicated that a neutral complex of ^{99m}Tc-citrate was formed. $^{99m}TcO_4^-$moved 18-

Figure 1A: 8 μm sections of turpentine-induced abscess, showing from inside out; abscess cavity, fibrin formation and migration of leukocytes into interstitial space (x115).



20 cm towards anode in all the buffer systems tested. No $^{99m}TcO_4^-$ was detected in the prepared mixtures. Only a small fraction (0.343±0.107%) of ^{99m}Tc-citrate was extractable into n-butanol. A highly water soluble complex was obtained as was also evidenced by its motility in saline on ITLC-SG strips. ^{99m}Tc-citrate was stable in plasma (only 2.16±0.71% $^{99m}TcO_4^-$), with low protein binding (18.06±0.45%) as determined by TCA precipitation. Binding to RBC's was also low (10.02±0.82%) and could be almost completely removed after 6 washings (Table 2).

In mice turpentine-induced abscesses were palpable easily on the 6th day. On dissection they measured about 1 cm in diameter. Staphylococcus induced abscesses were smaller (about 5-6 mm in diameter) at this time. In rabbits there was gross swelling around the affected joint, starting 48 h post-induction and continuing up to 21st day. Pathological findings confirmed the experimental observations. There was infiltration of leukocytes into the interstitial space in the abscess sections and under the synovial epithelium in arthritic knee joints, as evidenced by the photomicrographs in Figures 1 A- C.

The biodistribution of ^{99m}Tc-citrate in mice with trupentine- and Staphyloccocus-induced abscesses are given in Tables 3 and 4, respectively. The % uptake/g tissues in all the organs were low except for kidneys the only

Figure 1B: Staphylococcus induced abscess localized in the muscle (x30).

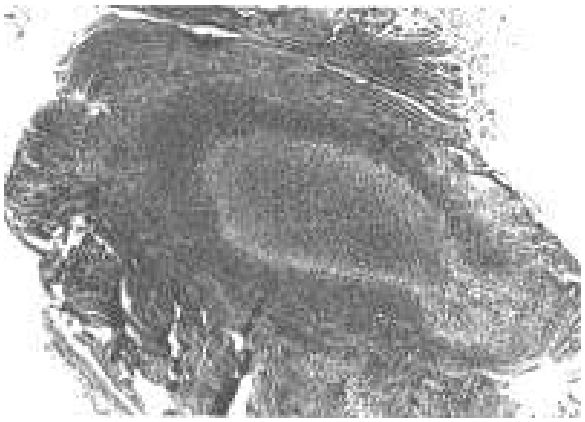
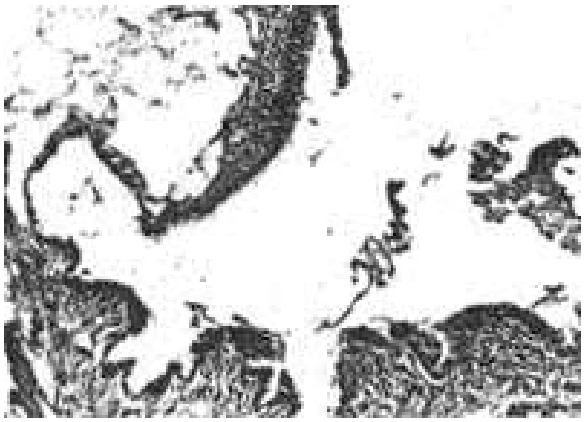


Table 3: Biodistribution of ^{99m}Tc-citrate in mice with turpentine-induced abscesses.

	% Uptake / g tissue *			
Organ	1 h	3 h	6 h	24 h
Blood	0.754 ± 0.304	0.413 ± 0.081	0.244 ± 0.0290	0.191 ± 0.0250
Liver	0.871 ± 0.171	0.502 ± 0.193	0.719 ± 0.1830	0353 ± 0.0940
Spleen	0.289 ± 0.083	0.199 ± 0.110	0.142 ± 0.0090	0.152 ± 0.0460
Stomach	0.605 ± 0.304	0.504 ± 0.175	0.513 ± 0.1940	0.170 ± 0.0480
Heart	0.393 ± 0.076	0.199 ± 0.085	0.129 ± 0.0180	0.112 ± 0.0260
Lungs	1.01 ± 0.330	0.325 ± 0.112	0.235 ± 0.0200	0.200 ± 0.0490
Intestines	1.11 ± 0.150	0.740 ± 0.334	0.903 ± 0.1280	0.238 ± 0.0380
Pancreas	0.430 ± 0.151	0.297 ± 0.252	0.170 ± 0.0590	0.113 ± 0.0290
Kidneys	5.94 ± 0.260	4.67 ± 2.390	3.62 ± 0.4800	3.59 ± 1.1600
Abscess	1.25 ± 0.190	0.423 ± 0.174	0.224 ± 0.0230	0.277 ± 0.0920
Muscle	0.300 ± 0.197	0.104 ± 0.012	0.0676 ± 0.0095	0.0755 ± 0.102
Urine	192.6 ± 99.800	65.03 ± 75.240	5.01 ± 1.3100	

* All values are mean±s.d.

Figure 1C: 21-day-old ovalbumin- induced arthritis demonstrating exudates in the articular cavity and infiltration of inflammatory cells under the synovial epithelium (x115).



organs with appreciable amounts of radioactivity. High levels of radioactivity in urine indicated excretion by kidneys. The distribution of ^{99m}Tc-citrate in organs was similar in both types of abscesses. The biodistribution of ^{67}Ga-citrate in mice with both abscess types is given in Table 5. It shows similarities to reported values in the literature (24) with organ uptakes which are clearly higher than the values obtained with ^{99m}Tc-citrate. The max. abscess/muscle (A/M) ratios for ^{99m}Tc-citrate were

Figure 2A: Scintigram obtained at 3 h post-injection of ^{99m}Tc-citrate.

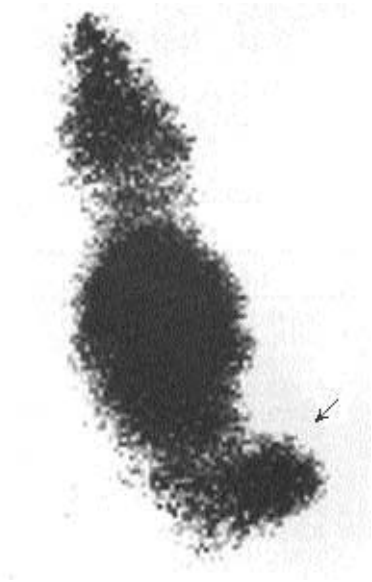
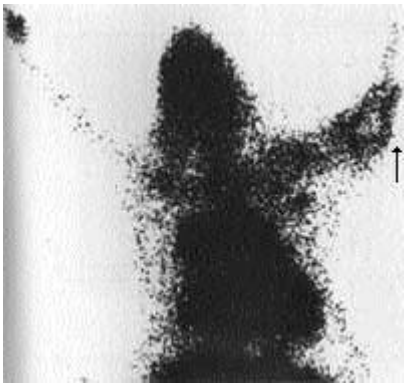


Figure 2B: Scintigram obtained at 4 h post-injection of ^{67}Ga-citrate in mice with turpentine induced 6-day-old abscesses in right thighs (arrows indicate abscesses. Note the similarity of uptake.

obtained at 1 h post-injection (4.00±1.39 and 4.47±1.76 for turpentine- and Staphyloccocus-induced abscesses). The A/M ratios remained at about the same level up to 24 h. Similar A/M ratios were obtained with ^{67}Ga-citrate ($p<0.50$). The max. abscesses/blood (A/B) ratios were 2.11±1.10 at 1 h and 4.19±2.05 at 6 h for turpentine- and Staphyloccocus-induced abscesses, respectively. The corresponding A/B ratios for ^{67}Ga-citrate were 2.79±1.08 and 1.21±044 at 24h. According to biodistribution studies in mice the scintigrams can be taken any time between 1-6 h post-injection of ^{99m}Tc-citrate.

The abscesses were well delineated on scintigrams up to 24 h. The best images were obtained at 3 h post-injection (Figure 2A), which were similar to ^{67}Ga-citrate images (Figure 2B). Scintigraphic images of rabbits demonstrated the arthritic knees very well with both agents (Figures 3A and B). However, there was a difference in the images; while ^{99m}Tc-citrate clearly demonstrated the synovial structures, ^{67}Ga-citrate remained in the surrounding inflammatory tissues. The max. A/C ratios obtained on scintigrams by ROI's over respective areas occurred at 3 h with a value of 2.68±0.87 for the turpentine- and at 6 h with a value of 3.49±1.09 for Staphyloccocus-induced

Figure 3A: Scintigram obtained at 3h post-injection of ^{99m}Tc-citrate.



abscesses. With ^{67}Ga-citrate the max. A/C ratios were 3.24±0.92 and 4.43±1.66, respectively. The max. arthritic/normal knee (A/N) ratios obtained by ROI's over respective areas on scintigrams taken at 96 h following the induction of arthritis were obtained at 24 h for ^{99m}Tc-citrate (1.95±0.55). It was considerably lower than the value (6.47±3.71) for ^{67}Ga-citrate. The A/C ratios increased as a function of age of abscess and of arthritis (Figure 4). The max. values were obtained between 6-10 days for turpentine-induced abscesses and 21 days for arthritic knee joints.

The blood clearance of ^{99m}Tc-citrate in 5 normal rabbits was biexponential (Figure 5). The clearance half-times were 51 min and 22 h for the slow and fast components, respectively.

Results of chromatographic and electrophoretic analyses of plasma and urine samples are summarized in Table 6. On ITLC the amount of free $^{99m}TcO_4^-$ was 2.16±0.71% in plasma and 1.60±0.30% in urine (Rf=1.0 in acetone), respectively. In plasma samples 44.1±4.6% of radioactivity remained at origin in saline. This might be the protein-bound fraction. In urine samples no such peak was observed. Almost all the activity migrated with the solvent front similar to prepared ^{99m}Tc-citrate samples (Table 1). On electrophoresis of plasma samples 3 peaks were obtained at 0.0 (29.4±15.3%), 8 (66.3%) and 20 cm 4.33±0.08%) towards anode, corresponding to ^{99m}Tc-citrate, protein-bound and $^{99m}TcO_4^-$, respectively. Variable and higher amounts of protein-bound fraction were obtained by the two methods compared to TCA precipitation. Electrophoretic analysis of urine samples showed only two peaks at 0.0 and 20 cm, indicating also that ^{99m}Tc-citrate was excreted intact without protein-binding or conjugation to another molecule. The amount of $^{99m}TcO_4^-$ was negligible (3.60±3.50%).

DISCUSSION

^{99m}Tc-citrate was found to be effective in the scintigraphic visualization of both types of abscesses and arthritis (Figures 2 and 3). In abscesses similar A/M and A/C ratios were obtained with ^{99m}Tc-citrate as with ^{67}Ga-citrate. The synovial structures were better demonstrated by ^{99m}Tc-citrate compared to ^{67}Ga-citrate in experimental arthritis. The biodistribution studies in mice (Tables 3 and 4) and scintigraphic studies in rabbits (Figure 3) indicated very low levels of organ uptakes except for kidneys and urinary bladder. This does not present a problem since the urinary bladder can be voided in clinical studies. High levels of liver uptake observed with ^{67}Ga-citrate was not detected with ^{99m}Tc-citrate. This is an advantage for the localization of abdominal abscesses. With ^{67}Ga-citrate the liver and the biliary tract are the main excretory route. Radioactivity accumulates in the stool and delayed studies up to 48 h or more are necessary (22). This problem was

Figure 3B : Scintigram obtained at 4 h post-injection of ^{67}Ga-citrate in rabbits with arthritis in their right front knees, induced 21 days previously (arrows indicate arthritic knees).

Figure 4: Abscess/contra-lateral tissue and arthritic/contra-lateral normal knee (A/C) ratios as a function of abscess or arthritis age, obtained at 3 h post-injection of ^{99m}Tc-citrate.

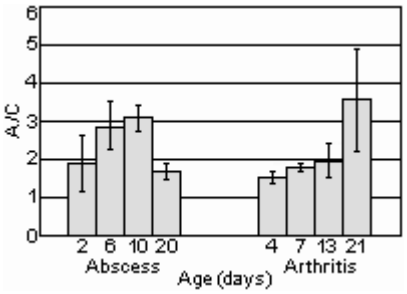
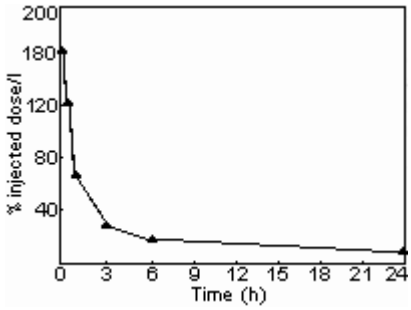


Figure 5: Blood clearance of ^{99m}Tc-citrate in normal rabbits, showing bi-exponential decrease of radioactivity.



reported also with ^{99m}Tc-HMPAO labeled leukocytes (6). Imaging with ^{99m}Tc-citrate can be performed as early as 1 h post-injection. While ^{99m}Tc-citrate clears rapidly from plasma with low RBC's and protein binding (Table 2), ^{67}Ga binds rapidly to transferring in plasma and is generally distributed within the body. High blood radioactivity levels observed with ^{67}Ga-citrate at 4 h persisted up to 24 h post-injection (Table 5). In addition, ^{99m}Tc is an ideal radio nuclide with a single photo peak at 140 keV and a physical half-life of 6 h. ^{67}Ga has unfavorable physical characteristics of a long physical half-life of 3.2 days and multiple gamma energies with some difficulty in effective collimation and high radiation burden to patients. Although the very high A/M and A/B concentration ratios obtained with ^{111}In-leukocytes by McAfee *et al.* (13) was not attained by ^{99m}Tc-citrate, in view of the disadvantages of cell harvesting and in vitro labeling of leukocytes, requiring time-consuming steps, ^{99m}Tc-citrate offers an easy method of localizing inflammatory lesions and deserves to be evaluated in clinical studies to show its sensitivity and specificity. ^{99m}Tc-citrate is inexpensive and both ^{99m}Tc and citric acid are widely available. It can be prepared in-house by a simple and rapid procedure that can be also put in a kit formulation.

The main mechanism of ^{99m}Tc-citrate localization might be infiltration into interstitial space due to increased

Table 4: Biodistiribution of ^{99m}Tc-citrate in mice with Staphylococcus aureus (ATCC 25923)-induced abscesses.

	% Uptake / g tissue *			
Organ	1 h	3 h	6 h	24 h
Blood	0.672 ± 0.285	0.356 ± 0.074	0.224 ± 0.110	0.168 ± 0.012
Liver	1.21 ± 0.26	0.615 ± 0.124	0.573 ± 0.142	0.332 ± 0.005
Spleen	0.186 ± 0.035	0.157 ± 0.019	0.161 ± 0.029	0.242 ± 0.045
Stomach	0.825 ± 0.820	0.424 ± 0.181	0.480 ± 0.195	0.241 ± 0.059
Heart	0.301 ± 0.041	0.325 ± 0.126	0.380 ± 0.315	0.164 ± 0.041
Lungs	0.585 ± 0.057	0.374 ± 0.114	0.380 ± 0.063	0.245 ± 0.008
Intestines	0.687 ± 0.162	0.456 ± 0.122	0.908 ± 0.183	0.244 ± 0.066
Pancreas	0.289 ± 0.037	0.214 ± 0.127	0.224 ± 0.034	0.184 ± 0.094
Kidneys	6.48 ± 1.30	3.03 ± 1.46	3.63 ± 0.52	1.19 ± 0.14
Abscess	0.906 ± 0.430	0.807 ± 0.239	0.938 ± 0.446	0.528 ± 0.142
Muscle	0.198 ± 0.026	0.228 ± 0.174	0.245 ± 0.114	0.200 ± 0.067
Urine	58.3 ± 27.3	35.3 ± 26.1	+40.1	

* All values are means ±s.d.

\+ Only one urine sample.

capillary permeability at the site of inflammation, as in the case of a lot of other agents, including ^{67}Ga-citrate and labeled proteins (24). After reaching a peak concentration ^{99m}Tc, citrate might leak out, due to its low molecular weight and low protein-binding, and is removed from circulation rapidly. In our studies elution of radioactivity from the target sites was observed as time progressed (Tables 3 and 4). The localization of compounds in synovial fluid depends also on their molecular weight (5). The image obtained with ^{99m}Tc-citrate indicated that it has diffused into the synovial fluid. This can be attributed to its low molecular weight and low protein binding. On the other hand, ^{67}Ga is known to bind to transferring in plasma (24), thus assuming a larger molecular weight. It also indicates the absence of an additional mechanism of localization. The accumulation of a similar compound, ^{99m}Tc-glucoheptonate, in ocular inflammation in rabbits was recently reported (19). The localization mechanism was thought to be simple diffusion through injured capillaries and uptake of inflammatory cells of glucoheptonate, a glucose analog, for utilization as an energy source. This additional mechanism might be in operation and apparently plays an important role in its uptake by tumoral tissues as well. However, it is doubtful whether glucoheptonate retains its biological properties in a metal complex structure. Since no additional mechanism is foreseen for ^{99m}Tc-citrate comparative studies can be performed to clarify this point.

Table 6: Results analysis of plasma and urine samples obtained from mice by ITLC and electrophoresis (E).

Sample	Method	Medium	Rf (ITLC) or Distance (cm) towards anode (E)	%
Plasma	ITLC	Acetone	0.0	97.8±0.7
			1.0	2.16
		Saliene	0.0	44.1±4.6
			1.0	55.9
Urine		Acetone	0.0	98.3±0.3
			1.0	1.60
		Saliene	0.0	2.44
			1.0	97.6±1.4
Plasma	E	Veronal buffer	0.0 cm	29.4±15.3
			8	66.3
			20	4.33±0.08
Urine			0.0	96.4±3.50
			20	3.60

In conclusion, ^{99m}Tc-citrate was prepared in a simple and rapid procedure which can also be put into a kit form. It was stable both in vitro and in vivo with low erythrocyte and protein binding, fast blood clearance and excretion mainly through the kidneys. Its accumulation in experimentally induced abscesses and arthritis was sufficient for scintigraphic visualization. It is preferred to ^{67}Ga-citrate, due to the ideal physical characteristics of ^{99m}Tc, easy preparation method, low cost, early accumulation in inflam-

Table 5: Biodistribution of ^{67}Ga-citrate in mice with abscesses.

	% Uptake / g tissue *			
	Turpentine		Staphyloccocus	
Organ	4 h	24 h	4 h	24 h
Blood	4.80 ± 1.98	1.48 ± 0.31	6.47 ± 0.84	3.60 ± 0.64
Liver	4.72 ± 0.94	8.81 ± 1.33	3.84 ± 0.17	4.30 ± 0.26
Spleen	3.20 ± 0.87	6.56 ± 2.49	3.09 ± 0.36	3.08 ± 0.25
Stomach	2.10 ± 0.41	2.17 ± 0.75	2.43 ± 0.07	2.46 ± 0.08
Heart	4.26 ± 1.85	1.40 ± 0.18	3.06 ± 0.64	3.05 ± 0.65
Lungs	6.15 ± 1.32	2.56 ± 0.35	5.23 ± 1.51	5.34 ± 1.63
Intestines	4.35 ± 1.31	3.54 ± 0.39	3.13 ± 0.56	3.33 ± 0.45
Pancreas	2.86 ± 1.00	1.61 ± 0.30	2.47 ± 1.14	2.37 ± 0.23
Kidneys	6.67 ± 1.39	9.14 ± 3.08	4.38 ± 0.44	4.45 ± 0.42
Abscess	4.76 ± 1.05	4.12 ± 1.00	4.33 ± 2.02	4.34 ± 2.06
Muscle	1.07 ± 0.18	0.90 ± 0.20	1.09 ± 1.05	1.12 ± 1.06
Urine	7.60 ± 0.11	3.31 ± 1.59	3.76 ± 0.11	3.64 ± 0.15

* All values are means ±s.d.

matory lesions and preference of renal rather than the biliary route of excretion, thus the absence of radioactivity in the abdominal organs.

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