EVALUATION OF TECHNETIUM-99m LABELED DEXTRAN FOR THE VISUALIZATION OF EXPERIMENTAL ABSCESSES: A COMPARATIVE STUDY

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SUMMARY : ^{99m}Tc-dextran was evaluated for the scintigraphic visualization of abscesses in comparison to ^{99m}Tc-HIG. Twenty-four mice were injected with 50 µl turpentine in left thigh muscle. Six days later they were injected with either radio pharmaceutical (RP) containing 15 MBq ^{99m}Tc. They were sacrificed at 1, 3, 6 and 24 h and scintigrams were obtained. All the organs, the whole abscess, some blood and urine were removed, weighed and counted in a gamma counter. % injected dose/g and the concentration ratios were calculated. Blood clearance of both RP's was determined in 10 normal rabbits. The biodistribution and blood clearance studies showed similarities between the two RP's. The highest accumulation was observed in the blood, liver and kidneys with some excretion in the urine. The maximum abscess/muscle ratios were 5.37±0.67 and 5.98±1.17 at 24 h with ^{99m}Tc-dextran and ^{99m}Tc-HIG, respectively. Both RP's had prolonged blood clearance. The abscesses were well visualized with both RP's but high blood background was evident. The localization mechanism of ^{99m}Tc-dextran was thought to be due to increased capillary permeability, possible binding to proteins at the site of inflammation and difficulty in back-diffusion into blood due to high molecular weight. It is preferred to ^{99m}Tc-HIG due to lower cost and easy in-house preparation.

Key Words : ^{99m}Tc-dextran, ^{99m}Tc-HIG, inflammation.

INTRODUCTION

Although many radio pharmaceuticals (RP's) have been introduced for the scintigraphic visualization of inflammatory lesions (2,4-11,13-16,20,23-28,30), none of them has been accepted for routine clinical use due to various shortcomings associated with each RP as fully discussed in current literature (1,12,17,18,21,22,28). All these RP's can be classified as labeled; 1) nanometer-sized particles or liposomes (2,20), 2) macro-molecules such as proteins (HSA, HIG, antibodies) (24,28), 3) small molecular weight compounds (citrate, glucoheptonate, DTPA, TcO_4, GSH, etc.) (4-9,11,23) and ^{99m}Tc or ^{111}In labeled leukocytes (27). The only RP 100% specific for inflammation is the labeled leukocytes (19) which have very high concentration ratios not attained so far by any other RP's. The rest is nonspecific and accumulate in tumoral tissues as well (14,25,28). In vitro labeling of leukocytes brings out some problems such as contamination and require trained personnel for handling patient's blood, cell harvesting and labeling (17). High cost of ^{111}In or HMPAO kits used for

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^{99m}Tc labeling has also to be considered. In vivo labeled leukocytes has other shortcomings of labeling other blood components and of bone marrow localization (12,15). Although the ultimate aim is to achieve 100% specificity with agents that are easily prepared, directly administered and scintigrams obtained in a few hours post-injection with high target to non-target ratios, none of the presently available RP's meet all these requirements and the search for the ideal agent continues.

^{99m}Tc labeled immunogamma globulin (HIG) initially proposed as a specific agent for inflammation (24) was later demonstrated to be nonspecific (25,28). The commercially available kits are highly expensive and as such their widespread use in clinics is hindered. In the present study ^{99m}Tc-dextran, also a macro-molecule like HIG, was evaluated in mice with turpentine-induced abscesses in comparison ^{99m}Tc-HIG with the hope of obtaining a better RP for imaging inflammation and to shed some light on localization mechanism.

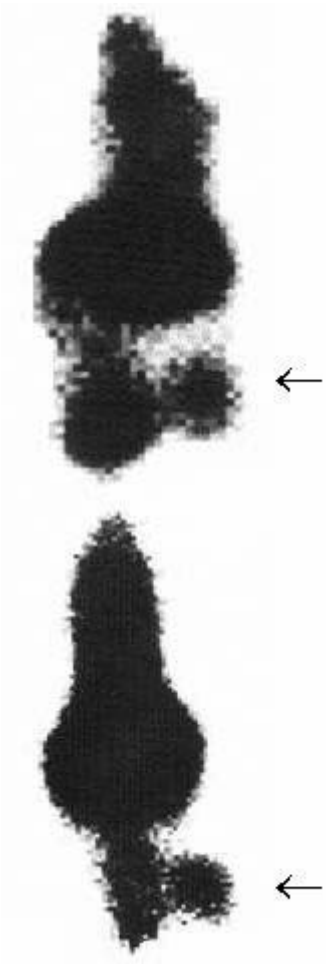
Table 1: Biodistribution of ^{99m}Tc-dextran in mice with turpentine induced abscesses.

Organ	% Uptake/g tissue*			
	1 h	3 h	6 h	24 h
Blood	4.54±1.91	2.76±0.81	1.65±0.27	0.894±0.122
Liver	13.04±0.67	9.82±0.47	6.12±2.30	5.03±0.69
Spleen	2.32±0.31	2.19±0.79	1.30±0.05	1.15±0.06
Stomach	0.521±0.090	0.600±0.321	0.790±0.128	0.478±0.081
Heart	1.51±0.24	1.11±0.45	0.855±0.010	0.489±0.093
Lungs	3.91±0.47	2.22±0.63	1.91±0.40	0.900±0.152
Intestines	1.41±0.27	1.91±0.49	1.81±0.59	0.646±0.146
Pancreas	1.33±0.29	0.899±0.253	1.12±0.18	0.420±0.102
Kidneys	6.31±0.57	5.80±2.60	4.61±0.95	2.79±0.61
Abscess	1.31±0.41	1.23±0.26	1.01±0.41	0.832±0.093
Muscle	0.567±0.081	0.403±0.156	0.318±0.046	0.148±0.022
Urine	149.0±7.2	44.0**	30.9±16.5	9.32**

* All values are mean±S.D.

** Only one urine sample.

Figure 1: Static images obtained in mice at 3 h post-injection of A) ^{99m}Tc-dextran and B) ^{99m}Tc-HIG demonstrate the abscesses (arrows) very well.



MATERIALS AND METHODS

^{99m}Tc generator was obtained from Amersham International, Amersham, UK. HIG kits were obtained from Mallinckrodt Medical B.V., Holland. They were labeled with ^{99m}Tc according to the instructions supplied by the manufacturer. Dextran (Ave. M.W=60000-90000 was purchased from Sigma Cem. Co., U.S.A. It was labeled with ^{99m}Tc by stannous chloride reduction method according to previously published procedure (3). The labeling efficiencies were determined at 15 min after preparation by the use of ITLC-SG (Gelman Instruments Co., U.S.A.) mini strips using acetone as a solvent as described before (3, 6).

Animal Studies

The animal studies were carried out in accordance with the

Table 2: Abscess/other tissue concentration ratios obtained from biodistribution of ^{99m}Tc-dextran in mice with turpentine induced abscesses.

	1 h	3 h	6 h	24 h
Abscess/liver	0.0993±0.0255	0.127±0.033	0.161±0.006	0.169±0.034
Abscess/blood	0.339±0.132	0.459±0.041	0.666±0.359	0.935±0.049
Abscess/muscle	2.27±0.41	3.30±0.63	3.04±0.86	5.37±0.67
Abscess/intestines	0.920±0.118	0.654±0.033	0.535±0.054	1.33±0.20
Abscess/kidney	0.205±0.049	0.240±0.063	0.208±0.046	0.307±0.042

British animal protection practice (4). Turpentine-induced abscesses were produced in 24 Swiss albino mice weighing 20-25 g by injection of 50 μl turpentine into the right thigh muscle. The biodistribution studies were carried out when the abscess age was 6 days (4). 12 mice were injected with 15 MBq ^{99m}Tc-dextran 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 liver, spleen, stomach, heart, lungs, intestines, pancreas, kidneys, the whole abscess and some skeletal suckle 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 a gamma counter (Berthold, BF 5300, Germany) 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) to obtain tissue concentration ratios. The same procedure was followed with ^{99m}Tc-HIG as well.

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

Organ	% Uptake/g tissue*			
	1 h	3 h	6 h	24 h
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**	-	-

* All values are mean±S.D.

** Only one urine sample.

Table 4: Abscess/other tissue concentration ratios obtained from biodistribution of ^{99m}Tc-HIG in mice with turpentine induced abscesses.

	1 h	3 h	6 h	24 h
Abscess/liver	0.187±0.027	0.251±0.010	0.450±0.042	0.995±0.144
Abscess/blood	0.159±0.037	0.233±0.038	0.591±0.033	0.914±0.351
Abscess/muscle	2.67±0.15	3.37±0.61	3.62±0.86	5.98±1.17
Abscess/intestines	0.749±0.089	0.708±0.204	0.637±0.080	1.88±0.42
Abscess/kidney	0.102±0.009	0.126±0.013	0.130±0.008	0.148±0.083

Blood clearance of both of the RP's was studied in 10 normal rabbits weighing 2-3 kg. 37 MBq of each agent in 1 ml was injected through the ear vein in 5 rabbits. Blood samples 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/l were plotted as a function of time.

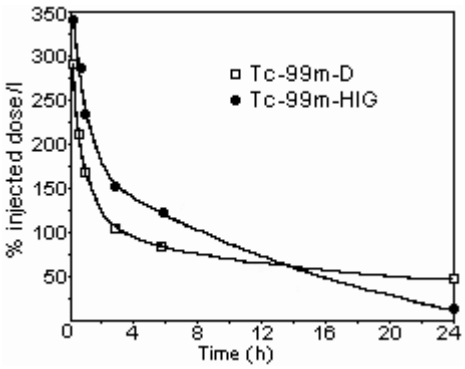
Pathology

Two mice with turpentine-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 section were taken. They were stained with haematoxylin and eosin.

RESULTS

Both RP's were prepared with high labeling efficiency (>99%) as determined by ITLC. The biodistribution ^{99m}Tc-dextran in mice with turpentine-induced abscesses is presented in Table 1 and the concentration ratios in Table 2. The maximum accumulation was observed in the liver, followed by the kidneys. Blood radioactivity in the urine indicated excretion of ^{99m}Tc-

Figure 2: Blood clearance of ^{99m}Tc-dextran and ^{99m}Tc-HIG in normal rabbits.



dextran by the kidneys. All the organ uptakes decreased as time progressed except for stomach and intestines which showed a slight increase due to free pertechnetate. Abscess to other organ ratios were lower than 1 except for A/M which reached a maximum value of 5.37±0.67 at 24 h. The biodistribution of ^{99m}Tc-HIG in mice with turpentine-induced abscesses is presented in Table 3 and the concentration ratios in Table 4. There are similarities in tissue uptake values between the 2 RP's. Again the blood, liver and kidneys showed highest accumulation. Radioactivity in the urine indicated renal excretion. The tissue concentration ratios were almost the same in both RP's.

The abscesses could be well visualized on scintigrams by both RP's. High blood pool, liver and kidney accumulation were also evident (Figure 1).

The blood clearance of both RP's in normal rabbits is displayed in Figure 2. The clearance of ^{99m}Tc-dextran was faster at the beginning compared to ^{99m}Tc-HIG, reaching a plateau at 6 h post-injection, while the decline in ^{99m}Tc-HIG continued up to 24 h.

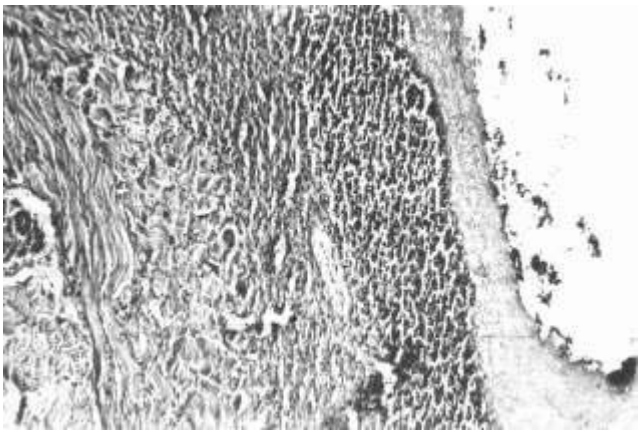
The pathological findings confirmed the formation of abscesses as evidenced by the photomicrographs which showed the migration of leukocytes into the interstitial space (Figure 3).

DISCUSSION

Our results demonstrated that ^{99m}Tc-dextran is as effective as ^{99m}Tc-HIG in the visualization of experimental abscesses in mice. This indicates the nonspecific nature of accumulation of these complexes in abscesses. A lot of ^{99m}Tc complexes have been tested previously for this purpose and showed accumulation in experimental abscesses. This included macromolecules such as proteins (HSA, antibodies, etc.) as well as small molecular weight compounds such as DTPA, citrate, pertechnetate, etc. (8,11,28) which have no biological significance. Dextran is a high molecular weight compound (M.W.=60000-90000) comparable to proteins. It accumulates in the liver where dextran is oxidized to lower molecular weight fractions, consisting of dextrose units that are then excreted by the kidneys (3). The metabolized fractions of dextran might also accumulate in the abscesses as well as intact dextran. However, the maximum A/M concentration ratio attained after 24 h is attained about the same as and not greater than that obtained with ^{99m}Tc-HIG (Tables 2 and 4).

Both dextran and HIG, being macromolecules, have prolonged blood clearance. This is a disadvantage in the identification of abscesses against high blood background on scintigrams. Another disadvantage is their accumulation in the liver and kidneys which renders the visualization of abdominal abscesses difficult. In this respect small molecular weight complexes of ^{99m}Tc that have rapid blood clearance via kidneys without any significant uptake by other organs and tissues (5,9) should be preferred to macromolecules. Labeled peptides are proposed (9,10) to overcome some of the problems encounted with proteins or substances which have high plasma protein binding such as ^{67}Ga (28). An ideal radio pharmaceutical for imaging inflammation should have rapid blood clearance with low protein binding, preference of renal rather than the biliary route of excretion, no significant uptake by other organs or tissues and high target-to-non-target ratios attainable in a few hours following administration (8,10,25). Neither ^{99m}Tc-dextran nor ^{99m}Tc-HIG meet these requirements completely. Although not an ideal RP, cold kits of HIG are commercially available, but they are highly expensive, preventing their wide-spread use in routine practice. On the other hand, ^{99m}Tc-dextran can be easily prepared in-house at a very low cost.

Figure 3: 8 μm sections of turpentine induced abscess, snowing from right to left the abscess cavity, fibrin formation and migration of leukocytes into interstitial space (x115).



We believe that increased capillary permeability is the main underlying mechanisms of localization in abscesses for both of the RP's. However, the increase in concentration ratios at 24 h post-injection of ^{99m}Tc-dextran or ^{99m}Tc-HIG (Tables 2 and 4) suggest that an additional mechanism might be in operation with these complexes or their metabolites as in the case of ^{99m}Tc-dextran. They are either glucose analogs or proteins that have biological significance. This additional mechanism might be specific or aspecific binding to proteins at the sites of inflammation after they leak out through the injured capillaries so that back diffusion into the blood is hindered (8). It can also be explained by high molecular weights of these complexes (11). Back diffusion into blood must be faster with small molecular weight compounds as indicated by their observed wash-out in concentration ratios (5,6).

In conclusion, ^{99m}Tc-dextran is as good as ^{99m}Tc-HIG for imaging inflammation. It has the advantage of lower cost and easy in-house preparation by a simple, rapid and reproducible procedure. It is nonspecific like ^{99m}Tc-HIG. It can be used in place of ^{99m}Tc-HIG until specific agents are found to replace them.

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