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Accumulation of ^{99m}Tc-Dextrans in Experimental Abscesses in Comparison to ^{67}Ga-Citrate

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Summary: *Two different molecular weight [(1) M.W. = 9300, (2) M.W. = 74000] dextrans were labelled with ^{99m}Tc and evaluated for the scintigraphic visualization of experimental abscesses in comparison to ^{67}Ga-citrate. 36 mice were injected with 50 µL turpentine in left thigh muscle. They were divided into 3 groups of 12 mice. 6 days later first group of animals was injected with 15 MBq of ^{99m}Tc-dextran (1), the second group with ^{99m}Tc-dextran(2) and the third group with 3.7 MBq of ^{67}Ga-citrate through the tail vein. The animals were sacrified in groups of 3 at 1, 3, 6 and 24 h. All the organs, the whole abscess, some blood and urine were removed, weighed and counted in a gamma counter. % injected dose/g organ and the concentration ratios were calculated. The highest uptake was observed in the liver, followed by kidneys and the urinary bladder with both dextrans. The maximum A/M ratios were 3.73±0.25(24 h), 5.37±0.67(24h) and 4.97±1.07(6h) for ^{99m}Tc-dextrans (1), (2) and 67Ga-citrate, respectively.*

Key words : ^{99m}Tc-Dextran, ^{67}Ga-citrate, experimental abscesses, inflammation

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^{99m}Tc-Dekstranların ^{67}Ga-sitrata göre Deneysel Abselerdeki Tutulumu

Özet: *Iki değişik molekül ağırlığındaki dextran [(1): M.A. = 9300, (2): M.A. = 74000] ^{99m}Tc ile işaretlendi. Deneysel abselerin sintigrafik olarak görüntülenmesi amacıyla ^{67}Ga-citrate ile mukayeseli olarak değerlendirildi. 36 farenin sol bacak adalesi içine 50 µL terebentin enjeksiyonu yapıldı. Fareler her biri 12'şerlik 3 gruba ayrıldı. 1. gruptaki farelere 15 MBq ^{99m}Tc-dekstran (1), 2. gruptakilere aynı miktar işaretli dekstran (2) ve 3. gruptakilere de 3.7 MBq ^{67}Ga-sitrat kuyruk veninden enjekte edildi. Fareler üçlü gruplar halinde 1, 3, 6 ve 24'üncü saatlerde öldürüldü. Bütün organlar, absenin tamamı, biraz kan ve idrar alındı, tartıldı ve radyoaktivitesi bir gama sayacında tespit edildi. Yüzde (%) enjekte edilen doz / gram organ veya doku konsantrasyon oranları hesaplandı. En fazla tutulum karaciğer, böbrekler ve mesanede görüldü. Maksimum abse/adale oranları dekstran (1) için 3.73±0.25 (24. saat), dekstran (2) için 5.37±0.67 (24. saat) ve ^{67}Ga-sitrat için 4.97±1.07 (6. saat) olduğu tespit edildi.*

Anahtar sözcükler : ^{99m}Tc-Dekstran, ^{67}Ga-sitrat, deneysel abseler, inflamasyon

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Introduction

Recent studies have indicated that both large and small molecular weight complexes of ^{99m}Tc accumulate in inflammatory lesions[1-8]. Although large molecular weight complexes have the advantage of higher absolute uptake by the inflammatory tissues because of prolonged blood clearance, they have high blood background, necessitating delayed studies. In addition, they accumulate in abdominal organs such as the liver and the kidneys, preventing easy identification of inflammatory lesions in this region. Small molecular weight complexes are predominantly excreted via kidneys with no specific uptake by any other organs, but lower absolute uptake by inflammatory tissues might present a sensitivity problem in scintigraphic detection of abscesses[3]. ^{67}Ga-citrate has been widely used in clinics due to its high sensitivity and considered to be a gold standard in imaging inflammatory lesions[9]. However, its localization in the liver and intestines as a result of its biliary excretion is a serious drawback. Moreover, ^{67}Ga has suboptimal physical characteristis of long physical half-life and multiple gamma rays of unsuitable energies for gamma cameras.

This study was undertaken to test the feasibility of imaging experimental abscesses with ^{99m}Tc labelled dextrans, which have large molecular weights. The results were compared to those obtained with ^{67}Ga-citrate.

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Materials and Methods

^{99m}Tc generator and ^{67}Ga-citrate were obtained from Amersham International, Amersham, U.K. Dextrans [(1) Ave. M. W. = 9300, (2) Ave. M. W. = 74000] were purchased from Sigma Chem. Co., U.S.A. They were labelled with ^{99m}Tc by stannous chloride reduction method according to a previously published procedure[10]. The labelling efficiencies were determined at 15 min after preparation by the use of impregnated thin-layer chromatography (ITLC) technique and ITLC-SG (Gelman Instrument Co., U. S.A.) mini strips using acetone as a solvent as described before[2,10].

Animal studies: The animal studies were carried out in accordance with the British animal protection practice[11]. Turpentine-induced abscesses were produced in 36 Swiss albino mice weighing 20-25 g by the injection of 50 µL turpentine into the right thigh muscle. The biodistribution studies were carried out when the abscess age was 6 days(1). 12 mice were injected with 15 MBq ^{99m}Tc-dextran (1) 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 were obtained by a gamma camera (Toshiba GCA 601 E), using a LEAP collimator. ROI's over abscesses and contralateral tissues were compared. The mice were dissected. The organs such as the liver, spleen, stomach, heart, lungs, intestines, pancreas, kidneys, the whole abscess and some skeletal muscle from the contralateral leg were removed. Blood and urine, when available were also obtained. The organs and tissues were weighed and counted at the photopeak 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 ^{99m}Tc-dextran (2). 12 mice were injected with 3.7 MBq ^{67}Ga-citrate in 0.1 mL and the above procedure was folowed, except that a medium energy collimator was used in obtaining scintigrams and that the tissues were counted with a wide window(200 keV) to include the two photopeaks (184 keV and 296 keV) of ^{67}Ga.

Results

Both dextrans (1) and (2) were labelled with ^{99m}Tc with high efficiency (>99 % labelling yield) as determined by ITLC. The biodistributions of ^{99m}Tc-dextan (1) and ^{99m}Tc-dextran (2) are presented in Tables 1 and 2, respectively. The biodistribution of ^{67}Ga-citrate is given in Table 3 for comparison. Both

Table 1. Biodistribution of ^{99m}Tc-dextran (1) in Mice with Turpentine Induced Abscesses

	% Uptake/g tissue (mean±SD)			
Organ	1h	3h	6h	24h
Blood	1.69±0.87	0.283±0.223	0.769±0.029	0.144±0.122
Liver	16.5±0.8	31.6±5.5	28.3±2.3	12.5±1.2
Spleen	1.90±0.61	1.61±0.50	1.17±0.11	1.03±0.29
Stomach	0.393±0.188	0.168±0.051	0.396±0.135	0.426±0.057
Heart	0.108±0.037	0.196±0.068	0.197±0.110	0.131±0.041
Lungs	1.81±0.42	0.822±0.100	0.700±0.165	0.612±0.154
Intestines	0.608±0.279	0.580±0.181	0.339±0.066	0.497±0.095
Pancreas	0.851±0.395	0.173±0.025	0.275±0.0621	0.062±0.037
Kidneys	1.55±0.73	1.56±0.48	3.24±0.04	3.34±0.22
Abscess	0.951±0.500	0.310±0.179	0.488±0.404	0.291±0.058
Muscle	0.384±0.200	0.170±0.016	0.183±0.054	0.078±0.067
Urine	1.21±0.75	16.7±8.2	7.66±2.03	6.74±1.31

Table 2. Biodistribution of ^{99m}Tc-dextran (2) in Mice with Turpentine Induced Abscesses

	% Uptake/g tissue (mean±SD)			
Organ	1h	3h	6h	24h
Blood	4.54±1.91	2.76±0.81	1.65±0.27	0.894±0.122
Liver	13.0±0.7	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±7	44.0*	30.9±16.5	9.32*

Table 3. Biodistribution of ^{67}Ga-citrate in Mice with Turpentine Induced Abscesses

	% Uptake/g tissue (mean±SD)			
Organ	1h	3h	6h	24h
Blood	6.46±1.31	4.57±0.77	1.41±0.45	0.926±0.175
Liver	5.86±0.58	7.58±2.13	6.72±1.48	7.04±0.70
Spleen	5.12±0.89	7.19±2.33	6.28±3.16	6.94±2.50
Stomach	1.36±0.45	2.50±1.21	2.94±1.03	2.01±0.38
Heart	4.30±0.56	3.93±0.44	3.08±0.51	1.49±0.26
Lungs	7.97±1.45	5.92±0.19	5.95±0.85	2.71±0.35
Intestines	2.66±0.25	4.04±0.85	4.02±1.39	2.23±0.16
Pancreas	3.34±0.82	2.77±0.22	2.01±0.24	1.73±0.25
Kidneys	5.91±0.78	5.87±1.73	7.83±1.39	8.44±0.87
Abscess	3.72±0.09	4.06±0.78	6.44±3.28	4.44±0.68
Muscle	2.27±0.16	2.47±0.63	1.68±1.20	0.924±0.154
Urine	7.71±0.90	4.48±0.03	24.4±17.0	1.41±0.19

dextrans accumulated mostly in the liver, followed by kidneys. High radioactivity levels in the urine indicated renal excretion. The accumulation in the liver was higher with dextran(1) compared to dextran(2), reaching maximum value at 3 h with 31.6±5.5 %/g, while dextran(2) showed an uptake of 13.0±0.7 %/g at 1 h and a slow decrease up to 24 h. The blood radioactivity levels were higher with dextran(2) compared to dextran(1), which can be attributed to its higher molecular weight and thus slower clearance from the blood. The uptake by other organs was much lower. The low radioactivity levels of stomach and intestines indicated the absence of pertechnetate and so the in vivo stability of ^{99m}Tc labelled dextrans and also the lack of biliary excretion. ^{67}Ga-citrate distributed almost homoge-

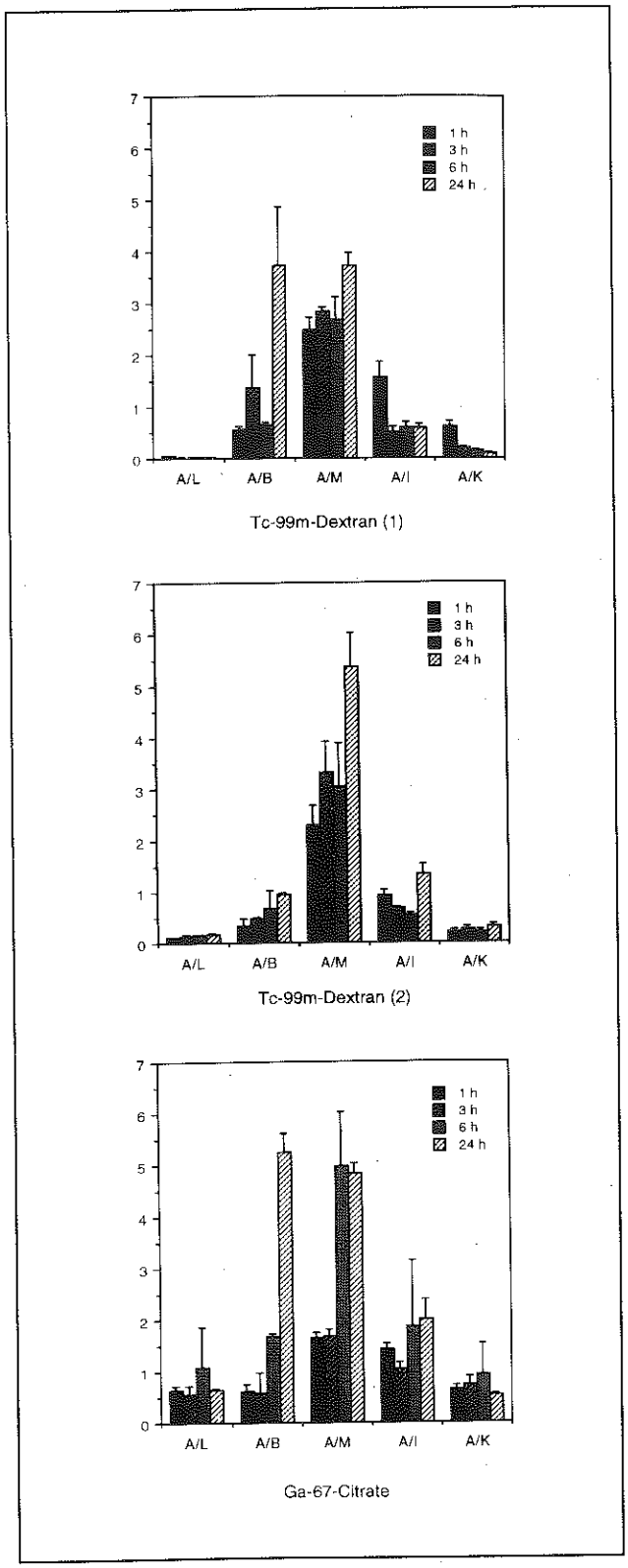

Figure 1. Abscess/liver (A/L), abscess/blood (A/B), abscess/muscle (A/M), abscess/intestine (A/I) and abscess/kidney (A/K) concentration ratios for A) ^{99m}Tc-dextran (1),B) ^{99m}Tc-dextran (2) and C) ^{67}Ga-citrate.

neously within all the organs and tissues with high blood levels in conformity with the previous reports[1, 7, 12].

The tissue concentration ratios are displayed in Figure 1 for all the radiopharmaceuticals. The ratios obtained with dextrans did not exceed the ^{67}Ga ratios. A/L, A/I and A/K ratios of ^{67}Ga were lower than those of 99mTc-dextrans. A/B ratios of dextran (1) was better at 24 h, reaching the level of A/M ratios. A/M ratios of dextran(2) were better than those of dextran(1) and the value at 24 h was almost the same as the value for ^{67}Ga-citrate.

The abscesses could be visualised on scintigrams by all the three radiopharmaceuticals. High blood pool, liver, kidney and urinary bladder activies were also evident (Fig. 2).

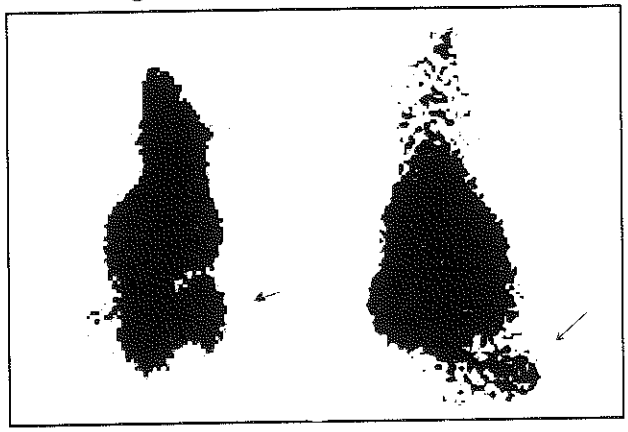

Figure 2. Scintigrams obtained in mice with turpentine-induced abscesses at 3 h post-injection of A) ^{99m}Tc-dextran (2) B) ^{67}Ga-citrate (arrows indicate abscessses).

Discussion

Our results demonstrated that experimental abscesses in mice could be visualised with ^{99m}Tc labelled dextrans and that higher molecular weight dextran(2) was better than dextran(1). However, ^{67}Ga-citrate was a little better than dextrans when the concentration ratios were compared (Fig. 1). Both ^{67}Ga-citrate and ^{99m}Tc-dextrans have the disadvantage of prolonged clearance from the blood and localization in abdominal organs such as the liver, kidneys and the urinary bladder. These are large molecular weight compounds or assumed to be of large molecular weight as in the case of ^{67}Ga-citrate from which ^{67}Ga dissociates and binds to

transferrin or lactoferrin in plasma[12]. The accumulation of other macromolecules such as proteins (human serum albumin, human immunogammaglobulin, etc.) labelled with suitable radionuclides in inflammatory lesions have also been reported with similar shortcomings[6,7]. The accumulation of dextran might also accumulate in the abscesses, as well as intact dextran in the liver, is well known[13]. It is oxidized in the liver to lower molecular weight fractions, consisting of dextrose units that are than excreted via the kidneys. The metabolized fractions of dextran might also accumulate in the abscesses as well as intact dextran, because these fractions are still labelled with ^{99m}Tc[3,14]. However, the maximum A/M ratio obtained after 24 h with dextran (2) is about the same as and not greater than that of ^{67}Ga-citrate. The main advantage of ^{99m}Tc-dextrans lies in the radionuclide, ^{99m}Tc, which is nearly ideal for imaging, because of its optimum physical characteristics ($T_{1/2}$= 6 h, Eγ = 140 kev), easy availability, and low cost.

We believe that increased capillary permeability is the main underlying mechanism of localization in inflammatory lesions for both of the dextrans, however the increase in concentration ratios at 24 h post-injection suggest that an additional mechanism might be in operation with these ^{99m}Tc complexes or their metabolites. ^{67}Ga is bound to transferrin in plasma and carried to the inflammaton site as such[12]. After they leak out through the injured capillaries, back diffusion into blood is hindered simply due to the large molecular weight of these agents or their protein bound fractions.

In conclusion, ^{99m}Tc-dextrans are not the ideal agents for the visualization of inflammatory lesions. However, the other radiopharmaceuticals such as ^{67}Ga-citrate and ^{99m}Tc-HIG used routinely for this purpose have similar shortcomings. Peptides labelled with an ideal radionuclide such as ^{99m}Tc might be better alternatives, because of their faster blood clearance, predominantly via kidneys and no significant accumulation by other organs or tissues. Future work should concentrate on small complexes of ^{99m}Tc rather than macromolecules.

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