Evaluation of ^{99m}Tc-Dextran for Blood-Pool-Imaging

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^{99m}Te-DEXTRAN'IN KAN HA VUZU ÇALIŞMALARI İÇİN DEĞERLENDİRİLMESİ

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SUMMARY

Dextran was labelled with ^{99m}mj-$_c$ and the binding efficiency was determined by mini-chromatography (Technetrol, Amersham, England). It was injected to four beagle dogs intra venously and samples of blood (at 5', 15', 30', lh, 3h, 6h and 24h) and urine (at 30', 3h and 24k) were obtained. The blood clearance and the affinity of ^{99m}Tc-Dextran to red blood cells were determined. Plasma and urine samples were analyzed by paper electrophoresis.

The labelling efficiency of ^{99m}Tc-Dextran was > 99% and stayed at this level up to 24h after preparation. Our results indicated a fast clearance of radioactivity from blood (~ 50% within lh). Most of the radioactivity in blood was in the plasma fraction (96%). Affinity of ^{99m}Tc-Dextran to red blood cells increased as time of sampling increased. In chromato-electrophoresis ^{99m}Tc-Dextran stayed at the point of application and ^{99m}TcO^ moved at a distance of 18-19 cm towards anode. In plasma and urine samples three peaks were obtained at 3-4, 6-7 and 11-14 cm with varying proportions of radioactivity. They were attributed to oxidized and broken-down fractions of dextran.

Our results indicated that ^{99m}Tc-Dextran is not an ideal radiopharmaceutical for blood-pool-imaging, but ^{99m}Tc-Dextrose migh be used to label red blood cells for the same purpose.

Key words: ^{99m}Tc-Dcxtran, ^{99m}Tc-Dcxtroic. blood clearance, electrophoresis

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ÖZET

Dextran ^{99m}Tc ile işaretlendi. Bağlanma miktarı mini-kromatografi metodu ile tayin edildi. İşaretli dextran intravenöz olarak 4 köpeğe zerkedüdL Kan numuneleri 5. 15, 30dak., 1, 3, 6 ve 24 saat sonra, idrar numuneleri de 30 dak., 3 ve 24 saat sonra toplandı. ^{99m}Tc-Dextrah'm kan Idirensi ile kırmızı kan hücrelerine olan afinitesi tayin edildi. Kan ve idrar numuneleri kağıt elektroforezi tekniği ile analiz edildi.

^{99m}Tc-Dextran'ın işaretlenme miktarı > % 99'du ve hazırlandıktan sonra 24. saate kadar bu seviyede kaldı. Bulgularımız radyoaktivitenin kandan hala temizlendiğini (1 saatte - % 50) gösterdi Kandaki radyoaktivitenin çoğu (% 96) plazma fraksiyonuna aitti. ^{99m}Tc-Dextran'ın kırmızı kan hücrelerine olan afinitesi numune alma zamanının artışıyla orantılı olarak arttı. Elektroforez çalışmalarında ^{99m}Tc-Dextran İlerleme göstermedi. ^{99m}Tc02 anoda doğru 18-19 cm ilerledi. Plazma ve idrar numunelerinde 3-4, 6-7 ve 11-14 cm'de olmak üzere muhtelif oranlarda radyoaktivite ihtiva eden 3 pik (maksimum) elde edildi. Bunlar dextranm oksitlenip küçük moleküllere ayrılmasından meydana gelen fraksiyonlar olarak değerlendirildi.

Bulgularımız ^{99m}Tc-Dextran'tn kan havuzu çalışmaları için ideal bir radyofarmasötik olmadığını, fakat ^{99m}Tc-Dextrose'un aynı amaçla kırmızı kan hücrelerini işaretlemede kullanıla bileceğini gösterdi.

Anahtar kelimeler: ^{99m}Tc-Dcxtran, ^{99m}Tc-Dextrose, kan klirensi, elektrofon:*

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Cardiac blood pool imaging using radioisotopes has become an important tool to study the cardiac function and anatomy. The information obtained in such images are useful in the diagnosis and treatment of cardiovascular disorders (4). The following properties are expected from a radiopharmaceutical for blood pool imaging: 1) The radiolabel should be stable, 2) It should stay in the vascular space for a

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long time, 3) The labelling procedure should be simple, fast and inexpensive and 4) It should have no harmful effects on the patients. A number of radiopharmaceuticals such as ^{99m}Tc labelled human serum albumin (Tc-HSA) or red blood cells labelled either in vitro or in vivo (Tc-RBC) have been developed for this purpose (6, 7). However, they are not considered ideal when these criteria are applied.

^{99m}Tc labelled dextran was proposed by Henze et al for both lymphoscintigraphy (3) and blood pool imaging (4). Previously we evaluated this radiopharmaceutical for ymphoscintigraphy both in experimental animals (2) and in clinical studies (1) and demonstrated that it is a promising agent for this purpose. For blood pool imaging Henze et al tested dextran with two different molecular sizes (M.W.: 40000 and 500000) in dogs and obtained adequate cardiac images up to 60 min following i.v. injection of dextran 500000. The image quality deteriorated starting at 30 min post-injection. Dextran (M.W.: 500000) cannot be used in clinics (3). In our study we used a lower molecular weight dextran (average M.W.: 82200, range: 60000-90000) to further evaluate this radiopharmaceutical. The size range preferred in this study is already used in clinics and can be safely administered in humans.

MATERIAL AND METHODS

1. Radiopharmaceutical Preparation and Quality Control:

Dextran (clinical grade, average molecular weight: 82200, range: 60000-90000) was purchased from Sigma Chemical Co., U.S.A. ^{99m}Tc was obtained from a generator (Squibb Medotopes Europa). The labelling procedure of Henze et al (4) was modified by us and reported in previous studies (1, 2).

Mini chromatography plates (Technetrol, Strips A) from Amersham, Buchler, England were used with 85% aq. methanol as solvent to determine the labelling efficiency. ^{99m}Tc-Dextran stayed at the point of application and free ^{99m}Tc04 impurity moved with the solvent front. In vitro stability of the radiopharmaceutical was tested at 10 min. 1h, 4h and 24h after preparation.

2. Animal Experiments:

a) Blood Clearance:

Four male Beagle dogs with a body weight of 12-16 kg were used without any premedication. 4-7 mCi ^{99m}Tc-Dextran in a volume of 2-3 ml was injected i.v. through the front leg. Heparinized blood samples, each containing at least 5 ml of blood, were withdrawn at 5', 15', 30', 1h, 3h, 6h and 24h. 1 ml of whole blood from each sample was counted in a 7-well-type counter (Berthold, model: BF 5300, F.R.G.) against a standard prepared from a 1/1000 dilution of the injected solution. The rest of the blood samples were centrifuged at 4000 rpm for 15 min. 1 ml plasma was separated from each and counted in the same manner.

b) Affinity of ^{99m}Tc-Dextran to Red Blood Cells:

Any remaining plasma over the erythrocytes in the blood samples obtained above was removed and discarded. These samples were counted against a ^{99m}Tc standard. The eels were washed with equal volumes of saline and centrifuged for 15 min at 4000 rpm. The saline was discarded and the cells were counted against the same standard. The washing procedure was repeated five times.

c) Radiochemical Analysis of Blood and Urine Samples:

Some plasma samples were saved for radiochemical analysis. Urine samples were collected at 30 min, 3h and 24h. Paper electrophoresis was performed on Whatman 3MM chromatography paper. 50 /tl of plasma and urine samples were spotted on separate strips (59 x 2 cm) of paper at a point 6 cm from one end. On separate strips ^{99m}Tc-Dextran and ^{99m}Tc04 were spotted as controls. They were all electrophoresed together in H.V. electrophoresis apparatus (Hormuth-Vetter, Pherograph, model: 64) in barbitone buffer (0.06 M, pH: 8.5) at 1000 V for 1h. Then the strips were removed, dried and cut into 1 cm segments and assayed for radioactivity in the automatic y-counter.

RESULTS AND DISCUSSION

The labelling efficiency of ^{99m}Tc-Dextran was > 99%. The amount of free ^{99m}Tc04 was always less than 1% up to 24h after preparation (Table -1).

Whole blood and plasma clearance curves are shown in Figure - 1. The disappearance of ^{99m}Tc-Dextran from blood is too fast to be of use for blood pool scintigraphy. The radioactivity in blood is mostly due to plasma activity (96.0 ± 1.8%). The absolute uptake of red blood cells is quite low (4.08).

The effect of washing the red blood cells with saline is shown in Figure - 2. At all the times studied after the initial washout, the radioactivity reached a plateau and no more radioactivity could be extracted other than negligible quantities. The retention of radioactivity by the erythrocytes increased as the time of blood sampling increased.

Electrophoretic analysis showed that ^{99m}Tc-Dextran stayed at the point of application with some streaking towards anode. Free pertechnetate **(TCO4)**

moved at a distance of 18-19 cm towards anode. In all the ^{99m}Tc-Dextran preparations and in plasma and urine samples no detectable $^{M m}$TcOJ was observed. Three peaks of radioactivity were obtained in plasma and urine samples. They appeared at 3-4, 6-7 and 11-14 cm towards anode and had varying proportions of radioactivity (Table - II). These three peaks are due to lower molecular weight dextrans They may contain 3 or more different molecular-sized fractions that have negative charge. Our results are consistent with the known metabolic breakdown of dextran which is oxidized in the liver and exreted by the kidneys (4).

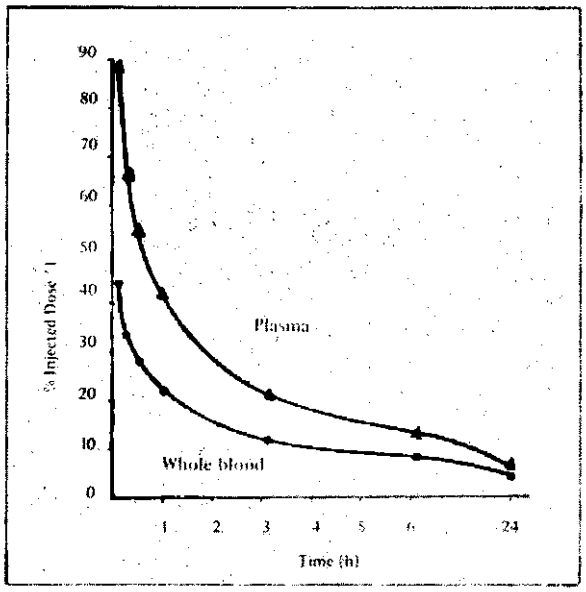

Figure 1. Whole blood and plasma clearance curves of ^{99m}Tc-Dextran in dogs.

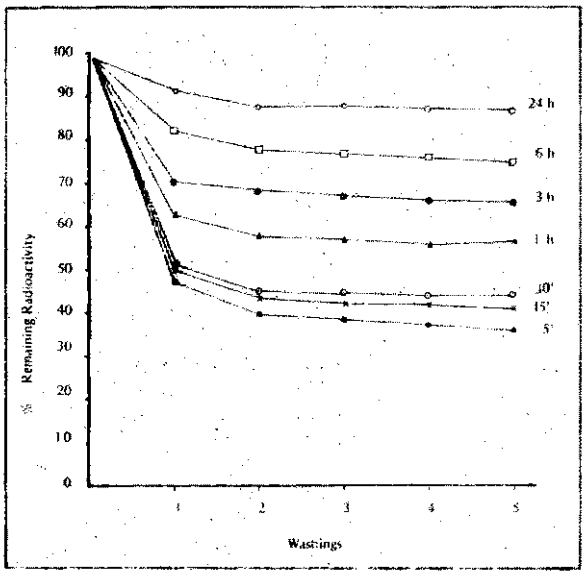

Figure 2. Effect of washing red blood cells with saline on the retention of radioactivity.

Table - 1

Stability of ^{99m}Tc-Dextran At Room Temperature Determined By Mini Chromatography (Technetrol) Using Strips (A) and 85% Aq. Methanol

Elapsed time after preparation	No. of determinations	Amount of free TcO_4^- (%)
10 min	15	0.35 ± 0.14
1 h	4	0.18 ± 0.04
4 h	6	0.37 ± 0.23
24 h	4	0.94 ± 0.46

Table — IF

Electrophoretic Mobilities of Various Radioactive Components Analyzed at 1000 V for 2 h Using Veronal Buffer (0.06 M, pH: 8.5)

^{99m}Tc labelled compound	Distance travelled towards anode (cm)
^{99m}Tc-Dextran	0
$^{99m}TcO_4^-$	18-19
^{99m}Tc-Dextran in plasma and urine	3-4, 6-7, 11-14

Our results indicate that Tc-to-dextern bond is very stable both in vitro (Table - I) and in vivo (Tab)o - II).

Henze et al (4) proposed dextran with a molecular weight dextrans, but it is too big for clinical application. Compared to red blood cell labelling either in vivo or in vitro, ^{99m}Tc-Dextran gave inferior scintigraphic images (4). ^{99m}Tc labelled RBC is the radiopharmaceutical of choice for blood pool studies at present, though there are some problems associated with the labelling procedure which needs improvement. In in vivo labelling procedures (6) the patient is i.v. given stannous pyrophosphate and 0.5b later the desired amount of ^{99m}TcO^. Organs such as thyroid, stomach and intestines compete with RBC's for the uptake of TcOi and as a result the labelling of RBC's are low. A modification of the method (8) was proposed where a volume of in vivo-tinned patient blood was incubated with pertechnetate in a closed in itro system before reinjection. Whatever the method used there is a side effect: long-term in vivo survival of stannous RBC's, which contraindicates the use of sodium pertechnetate as a radiopharmaceutical for several weeks (5). Mock and Wellman (5) introduced stannous EDTA labelling of RBC's in vitro and claimed its superiority over the previous methods, however, with this method there is the risk of con-

taminating the patient's blood since too many steps are involved in the labelling procedure.

Our results with $^{(})9m$Tc-Dextran indicate that it cannot be used successfully in blood pool imaging, because of its fast removal from the cardiovascular space, however the retention of radioactivity by RBC's is meaningful (Figure 2). Since the uptake of RBC's of the injected radioactivity is low (4%) compared to plasma activity (96%) the injected intact dextran or its oxidized but large molecular weight fractions are not taken up by RBC's. What is taken-up might be just a few molecular units or single units of dextrose still labelled with $^{9}{}^{9m}$Tc. If this is the case, then 99mTc-Dextrose may be effectively used to label RBC's either in vivo or in vitro for the same purpose. We plan to investigate this possibility in our laboratory in the near future.

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