^{99m}Tc-DEXTRAN-70: PREPARATION AND QUALITY CONTROL

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ABSTRACT

Dextran-70 labelled with ^{99m}Tc is used for lymphocintigraphy in Nuclear Medicine. The aims of this work were: the lyophilized kit formulation; the radiochemical quality control determination and the biodistribution studies in Wistar rats. Each lyophilized vial contains: 50 mg Dextran-70 (Sigma); 750 μg $SnCl_2.2H_2O$, pH = 4.0. For the radiochemical determination the following parameters were assayed: 1) Chromatography systems (Whatman 3MM, TLC-SG (Silica-gel) e TLC-Al (Aluminium); 2) the ^{99m}Tc activities (37, 111 and 1850 MBq); 3) the ^{99m}Tc volumes (1, 3, 5, and 8 mL) and 4) the stability after the lyophilization process (1, 3, 6, 12 and 24 months). The Whatman 3MM chromatography system using acetone as solvent presented a purity yield of 99.88; 99.70; 99.00 and 98.92 % using 1, 3, 5 and 8 mL of ^{99m}Tc, repectively. The yield of labelling showed 99.80 % of radiochemical purity using 1850 MBq of ^{99m}Tc, after 24 months. The biological studies were performed in Wistar rats, average weight 250g, after intravenous administration of ^{99m}Tc-Dextran-70 (2.96 MBq). A slow blood decrease with high hepatic uptake was measured. The high kidney uptake observed, during the experiment, was due the fact that the animals were kept under anaesthesic effect.

I. INTRODUCTION

^{99m}Tc-Dextran has been suggested as a promising agent for lymphocintigraphy in Nuclear Medicine and for noninvasive evaluation of cardiac function with gated equilibrium blood-pool imaging in clinical cardiology. Dextran has a long intravascular half-time and is used clinically as a plasma expander [1].

Henze (1982) has recommended purified Dextran of molecular weight between 40,000 and 70,000 to be labelled with ^{99m}Tc [2].

The tracer should: a) be stable during 3 - 4 hours, the radiolabel remaining tightly bound, b) remain in the vascular space, c) have a simple, fast and inexpensive labelling procedure and d) provide minimal risk to the patient. Dextran is a non colloidal and nonparticulate tracer compound, soluble in lymph fluid and with molecules large enough not to penetrate the capillary membrane after interstitial administration[3].

The purpose of this study was to formulate an instant lyophilized kit of stannous-Dextran-70 to be labelled with ^{99m}Tc, with the quality control methods (radiochemical and biological), for a further routine production.

II. MATERIALS AND METHODS

Materials

The Dextran used in this study was of molecular weight 70,000 obtained from Sigma and the hydrated stannous chloride was obtained from Nuclear with a 98 % of purity. The ^{99m}Tc solution was from IPEN-TEC generator.

Methods

Lyophilized kit formulation: The stannous-Dextran kits (Sn-Dex) were prepared in 30 units batches. To 1.5 g Dextran dissolved in 20 mL 0.9% NaCl was added 22,5mg of $SnCl_2..2H_20$ in 3 mL of 0.1 N HCl. Both solutions were maintained for 15 minutes under N_2 stream before use. The volume was adjusted to 30 mL with 0.9 % NaCl with a final pH = 4.0 and a final concentration of 500 mg of Dextran and 0.75 mg of $SnCl_2.2H_2O$ per mL. The whole misture was filtered through 0.22 μm membrane filter and under aseptic conditions aliquots of 1 mL were dispensed into sterile vials, and were lyophilized for 16 hours in the SuperModulyo Lyophilizator - Edwards. The vials were sealed and stored at 4° C.

Radiolabelling and Radiochemical Quality Control: Freshly eluted ^{99m}Tc was added to the vial, the solution was carefully mixed and left at room temperature for 15 minutes until the quality control tests. The radiochemical quality control was determined by: a) paper chromatography system (PC), Whatman 3 MM (8 x 1 cm), using 2 different solvents: 85 % methanol and acetone. b) TLC-SG and c) TLC-Al, both systems in the same solvents. Samples of 5 - 10 µl of ^{99m}Tc-Dextran-70 were spotted , dried under nitrogen stream and immediately developed on the solvents. Several parameters were studied: volume of ^{99m}Tc solution (1, 3, 5 and 8 mL); activities of ^{99m}Tc (37, 111 e 1850 MBq) and the kit stability after lyophilization (1, 3, 6, 12 and 24 months).

Biological Distribution: The biodistribution of ^{99m}Tc-Dextran-70 in Wistar rats (average weight 250 g) was studied by injecting 2.96 MBq intravenously. The animals were anesthetized with urethane (100 mg / kg weight) and then killed serially at 10, 30, 60 and 120 minutes after dose admistration.

The activities in different organs (liver, heart, kidney, spleen, intestine, stomach, liver, muscle and blood) were determined using a well-type scintillation counter (BERTHOLD) with the data normalized to the total administered radioactivity and corrected for physical decay of ^{99m}Tc. The results were calculated and expressed in % dose / organs or % dose / g.

III. RESULTS AND DISCUSSION

In the TLC-SG, TLC-Al and PC tests, the labelled Dextran remained at the origin (Rf = 0.0), while the free pertechnenate moved with the solvent front (Rf = 1.0), in both solvents. PC measuremens gave the same labelling efficiency than the others systems (more than 99.00%) for the different incubations time and parameters studied.

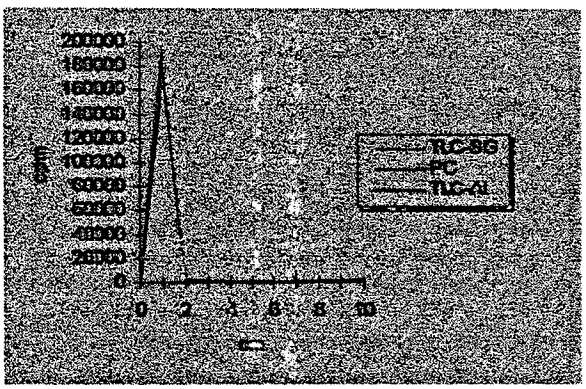

Figure 1. Radiochromatograms of ^{99m}Tc-Dextran-70, in acetone and, in 3 different systems (10 x cm)

When comparing the different quality control methods, the PC technique is a rapid method, easy and reproducible and do not need any complex or expensive equipment. Table 1 - Table 2.

TABLE 1. Radiochemical Purity (%) in PC using different ^{99m}Tc volumes

Time (min.) / Volume (mL)	1	3	5	8
15	99.50	99.83	99.79	99.47
60	99.51	99.90	99.60	99.35
120	99.53	99.83	99.78	99.58
240	99.52	99.72	99.70	99.18
300	99.98	99.88	99.76	99.03

TABLE 2. Radiochemical Purity (%) in PC using different ^{99m}Tc activities

Time (min) / Activity (MBq)	37	111	1850
15	99.97	99.60	99.58
60	99.95	99.98	99.80
120	99.97	99.87	99.34
240	99.97	99.86	97.80
300	99.89	99.87	97.26
360	99.87	99.59	97.18

TABLE 3. Radiochemical Purity (%) in PC (stalibity after lyophilization)

Time (min.) / Time (Month)	1	6	12	24
15	99.98	99.96	99.80	99.65
60	99.78	99.96	99.87	99.69
120	99.94	99.87	99.85	99.75
240	99.79	99.96	99.91	99.70
300	99.98	99.68	99.71	99.76
360	99.97	99.93	99.84	98.85

The highest labelling efficiencies were obtained in PC system after an incubation time of 15 minutes, the product showed an stability of 6 hours after labelling and 24 months after lyophilization (Table 3).

The biological distribution (Figure 2) demostrated no evidence of free Tc-99m after intravenous injection, as shown by the low uptake by the stomach, which presented 0.23 % dose / organ after 10 minutes (Table 4). Dextran with a molecular weight of less than 40,000 is excreted in man through the kidney. Henze (1982) related the urinary excretion rate in man from Dextran 70,000 up to 150,000

of about 40 % per 24 hours with a low blood clearance [2] . The blood uptake in the first stage (10 min.) was fast, followed by a slow clearance at 60 minutes, 22.72 % and 15.83 % dose / órgan, respectively. (Table 4)

The high uptake by kidney, 8.97 %dose / organ, in rats after 120 minutes of injection dose (Table 4) was due to the effect in part, that the animals were anesthetized during the time of the experiment [4], and / or by the elimination of Dextran of low molecular weight [2]. In this work the Dextran was 99% chemically pure.

TABLE 4. Biological Distribution in Wistar rats expressed in % dose organs

Organ / Time (min.)	10	30	60	120
Kidney	3.31	5.64	10.01	8.97
Stomach	0.23	0.28	0.35	0.22
Liver	17.04	16.95	16.19	15.08
Blood	22.72	15.67	15.83	9.73
Heart	0.33	0.27	0.30	0.22

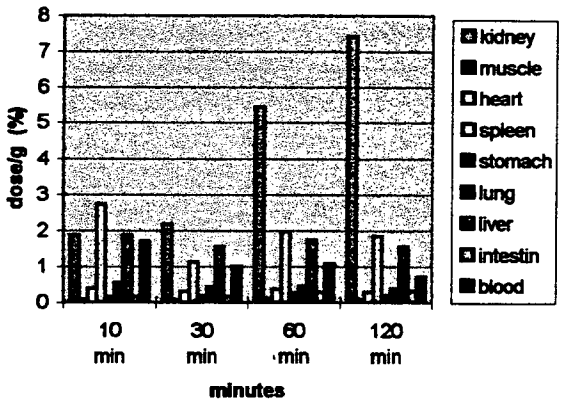

Figure 2. Biological Distribution in Wistar rats, expressed in % dose / g. (n=4)

V. CONCLUSION

This new noncolloidal and nonparticulate ^{99m}Tc-radiopharmaceutical (^{99m}Tc-Dextran-70) presented in this work, shows an easy and simple method of lyophilized kit formulation with high radiochemical purity. It promises to be a potencial agent for lymphoscintigraphy and for diagnosis and follow up of patients with cancer, lymphoma and primary lymphatic disease

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