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A FREEZE DRIED FORMULATION FOR THE PREPARATION OF ^{99m}Tc-[V]-DMS COMPLEX

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SUMMARY

A freeze dried kit formulation for the preparation of ^{99m}Tc-[V]-DMS complex was developed, requiring a single step labeling procedure. Methods of analysis were also developed permitting identification of radiochemical impurities which may be present in radiopharmaceutical solution. ^{99m}Tc-[V]-DMS prepared by the kit method was evaluated in experimental animals and human volunteers. Data demonstrated that the complex formed after kit reconstitution shows tumor seeking properties. Thyroid medullary carcinoma in patients was successfully detected 2 hrs post administration.

Key words: Dimercapto succinic acid DMS, ^{99m}Tc[V]-DMS complex, ^{99m}Tc-DMS kidney agent, ITL Chromatography, High Performance Liquid Chromatography HPLC.

INTRODUCTION

Dimercapto succinic acid coordinated with pentavalent technetium-99m developed by Yokoyama and col. (1) has been proved a potential radiopharmaceutical for the detection of certain types of tumor (2). Application of ^{99m}Tc-[V]-DMS to thyroid cancers resulted to the visualization of medullary carcinoma, where gallium-67 had only limited value (3,4). For the preparation of ^{99m}Tc-[V]-MS complex several methods have been proposed. These methods include either in situ preparations or a two step kit

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formulation [4,6].

In the present work we describe a convenient procedure for preparing ^{99m}Tc-[V]-DMS on the basis of a single composition instant freeze dried kit. The kit formulation was evaluated in experimental animals and human volunteers.

EXPERIMENTAL

Formulation of the kit

The preparation of the kit has been performed by mixing two solutions of dimercapto succinic acid, each one of different pH.

Solution A (acidic DMS solution): in 50 ml of water 25 mg of DMS, 17.5 mg ascorbic acid and 1.25 g inositol were dissolved under nitrogen atmosphere. Then 10.5 mg of tin chloride in acidic solution were added with stirring and the pH was adjusted to 2.2 with dilute hydrochloric acid.

Solution B (alkaline DMS solution): three g of dextrose and sodium bicarbonate (0.826 g) were diluted in 50 ml of water. DMS (111 mg) was then added under nitrogen atmosphere and the mixture was stirred until complete dissolution (final pH approx. 8.0).

Solutions A and B were then mixed in a beaker (final pH 7.8) and aliquots of 1 ml were dispensed in serum vials and lyophilized for 24 hrs. Vials were stoppered under dry nitrogen atmosphere and kept in the freezer until use.

Reconstitution of the kit was performed by adding 2.5 ml of pertechnetate (20-80 mCi) as eluted from the generator (CIS Int.). The radioactive solution was used within 2 hrs after reconstitution.

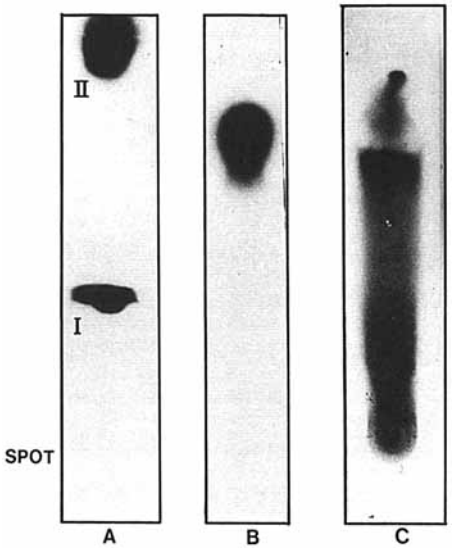

Fig. 1: Autoradiography patterns of ITL chromatograms obtained from analysis and characterization of ^{99m}Tc-[V]-DMS complex.
A: Silica gel. First run MEK: Pertechnetate Rf 0.9-1.0 (II). Second run glycine: ^{99m}Tc-[V]-DMS + ^{99m}Tc-DMS kidney agent Rf 0.9-1.0 (I). B,C: Silicic acid n-butanol:acetic acid:water 30:20:30, ^{99m}Tc-[V]-DMS Rf 0.8-0.9 (B), ^{99m}Tc-DMS kidney agent is resolved as a tail (C).

Radiochemical quality control

The yield of ^{99m}Tc-[V]-DMS complex was determined by instant thin layer chromatography (ITLC) and by high performance liquid chromatography (HPLC). In all studies ^{99m}Tc-DMS kidney agent prepared from a commercial kit (NRCPS "Demokritos", Greece) was used as the reference radiopharmaceutical. The following systems were used for analysis:

A. ITL Chromatography

- On silica gel strips (SG Gelman Chem. Co.):

First run: solvent methyl ethyl ketone (MEK), 20 cm developing distance.

Rf values 0.0 (spot) : ^{99m}Tc-[V]-DMS + ^{99m}Tc-DMS kidney agent + Reduced Hydrolyzed Tc-99m.

0.9-1.0 (front) : $^{99m}TcO_4^-$.

Second run: solvent glycine 5%, 10 cm developing distance.

Rf values 0.9-1.0 (front) ^{99m}Tc-[V]-DMS + ^{99m}Tc-DMS kidney agent.

0.0 (spot) Reduced Hydrolyzed Tc-99m.

- On silicic acid strips (SA Gelman Chem. Co.):

Solvent: n-butanol:acetic acid:water 30:20:30 (v/v)

Rf values 0.8-0.9 ^{99m}Tc-[V]-DMS

0.0-0.7 (tail) ^{99m}Tc-DMS kidney agent.

B. HPL Chromatography: (LDC/Milton Roy)

Column: Bondapak C18 (Waters Co.). Solvent acetonitrile: ammonium phosphate buffer (0.01 N) 20:80 (v/v) at a flow rate 1 ml/min.

Elution time: 2 min ^{99m}Tc-[V]-DMS (90% recovery of activity as a peak)

2.5 min $^{99m}TcO_4^-$

2-2.5 min ^{99m}Tc-DMS kidney agent (70% of activity was retained on the column).

Biodistribution studies

Organ distribution studies were performed in healthy mice and in rats bearing walker sarcoma. In white mice weighing 20-25 g the radiopharmaceuticals were injected through the tail vein, while in rats (weighing 150-200 g) through the femoral vein. Animals were sacrificed by ether in predetermined time intervals and the organs of interest were excised and counted in a scintillation counter in comparison with a standard.

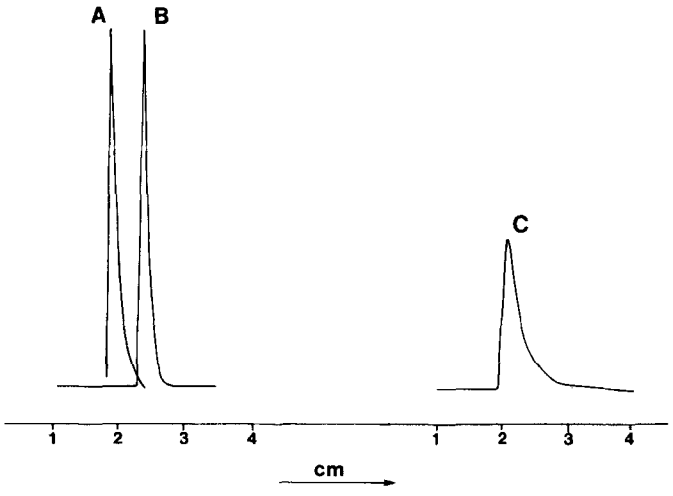

Fig. 2: HPLC analysis patterns. Solvent:acetonitrile:phosphate buffer 0.01 N, 20:80 (v/v), flow rate 1 ml/min. A: ^{99m}Tc-[V]-DMS. B: Pertechnetate. C: ^{99m}-Tc-DMS kidney agent.

Whole body scintigrams, of healthy rats and animals bearing the tumor, were performed 2 hrs after administration of ^{99m}Tc-[V]-DMS complex, using a small animal scanner (Berthold Co.).

RESULTS AND DISCUSSION

^{99m}Tc-[V]-DMS complex has shown tumor seeking properties and has been successfully used in the detection of thyroid medullary carcinoma [2,3]. Methods in preparation reported previously required the addition of 7% $NaHCO_3$ to pertechnetate prior reconstitution of kit ingredients [4]. Another method consisted of in situ preparation using a commercial kit of DMS kidney agent. In this kit sodium bicarbonate and a quantity of dimercaptosuccinate were added prior labeling with pertechnetate [6].

The present study demonstrated that this complex can be readily prepared on the basis of a single step instant freeze dried kit.

The method of kit formulation consisted in mixing two solutions of dimercapto succinic acid, one basic and the other acidic, under

nitrogen atmosphere. The final preparation contained stannous chloride as the reducing agent of Tc-99m and stabilizers such as ascorbic acid, inositol and dextrose. Labeling was performed simply by adding pertechnetate as eluted from the generator, in the kit vial.

Figures 1 and 2 demonstrate the radiochemical analysis of ^{99m}Tc-[V]-DMS prepared by the kit method. Autoradiography patterns of ITLC analysis using various solvent systems is presented in figure 1. Pertechnetate can be easily detected using ITLC silica gel strips and methyl ethyl ketone (MEK) as developing solvent. Free $^{99m}TcO_4^-$ is resolved in the solent front (fig. 1, A II), while ^{99m}Tc-[V]-DMS and ^{99m}Tc-DMS kidney agent stay at the origin.

A second run on the same chromatogram with solvent glycine 5% revealed ^{99m}Tc-[V]-DMS in the front (fig. 1, A I). ^{99m}Tc-DMS kidney agent is also moved to the front using glycine as developing solvent.

Table 1. Distribution in Mice* of ^{99m}Tc-[V]-DMS

	% Dose in organ				
	0.5 h	1 h	2 h	4 h	24 h
Blood	4.98	2.91	1.26	0.59	0.10
	(3.30-6.82)	(1.66-3.52)	(0.93-1.82)	(0.42-0.81)	(0.03-0.13)
Liver	2.27	1.64	1.02	0.92	0.30
	(1.46-3.12)	(0.99-1.97)	(0.73-1.25)	(0.77-1.25)	(0.22-0.45)
Kidneys	3.23	1.96	2.02	1.97	1.53
	(2.22-4.30)	(1.67-2.50)	(1.44-2.47)	(1.29-3.05)	(1.16-2.01)
Stomach	0.64	0.33	0.21	0.26	0.09
	(0.39-1.38)	(0.14-0.67)	(0.07-0.48)	(0.06-0.73)	(0.03-0.26)
Spleen	0.24	0.07	0.04	0.05	0.01
	(0.06-0.66)	(0.03-0.11)	(0.02-0.08)	(0.02-0.15)	(0.01-0.02)

Intestines	2.60	1.66	1.37	1.55	0.27
	(1.99-3.08)	(1.33-2.13)	(0.58-2.44)	(0.81-3.41)	(0.15-0.55)
Muscles	8.71	4.37	2.17	2.19	0.64
	(5.25-13.35)	(2.81-6.92)	(1.69-2.88)	(1.48-3.36)	(0.24-1.29)
Femur	0.62	0.56	0.47	0.51	0.16
	(0.37-1.03)	(0.27-0.90)	(0.32-0.97)	(0.15-1.20)	(0.10-0.27)
Tibia	0.48	0.65	0.47	0.47	0.17
	(0.25-0.86)	(0.50-0.76)	(0.23-1.07)	(0.15-0.93)	(0.11-0.24)
Total bone	12.93	15.25	15.17	14.08	5.30
	(8.91-18.99)	(13.37-17.25)	(9.80-25.47)	(9.21-21.68)	(2.62-11.08)
Urine	19.96	26.44	31.04	31.58	31.21
	(14.17-25.47)	(24.10-27.79)	(26.08-43.26)	(28.02-32.70)	(24.27-40.38)

values are the average of seven animals

^{99m}Tc-[V]-DMS complex was separated from ^{99m}Tc-DMS kidney complex by ITLC on silicic acid strips using n-butanol/acetic acid/water (30:20:30) as solvent system. The technetium tumor agent was moved as a distinct spot of Rf 0.8-0.9, while ^{99m}Tc-DMS kidney agent resolved on the chromatographic medium as a tail.

Representative analytical profiles of HPLC analysis using acetonitrile:phosphate buffer (0.01 N) at 1 ml/min flow rate are shown in Fig. 2.

^{99m}Tc-[V]-DMS was eluted with the void volume as a single peak (fig. 2,A). Pertechnetate was more retained and was eluted in 2.5 min as a separate peak. ^{99m}Tc-DMS kidney agent in the same system was mainly retained on the column and only 30% of the activity was recovered as a peak in 2-2.2 min (fig. 2,C).

The in vitro stability of the preparation of ^{99m}Tc-[V]-DMS was studied for 6 hrs after reconstitution, in 2 hrs intervals. No degradation of the radiopharmaceutical was observed within this period of time.

Table 2. Radiobiodistribution in walker sarcoma bearing rats*

% Injected dose per gram tissue

	^{99m}Tc-[V]-DMS (Tumor agent)		^{99m}Tc-DMS (kidney agent)	
	1h	2h	3h	3h
Blood	0.74±0.19	0.38±0.06	0.27±0.04	0.65±0.31
Liver	0.23±0.02	0.20±0.06	0.26±0.07	0.99±0.30
Kidneys	1.80±0.17	1.50±0.58	2.31±0.40	30.90±7.74
Stomach	0.09±0.05	0.05±0.03	0.02±0.02	0.04±0.00
Spleen	0.13±0.03	0.19±0.10	0.11±0.11	0.06±0.00
Intestines	0.12±0.03	0.18±0.12	0.35±0.11	0.23±0.10
Muscle	0.09±0.03	0.08±0.05	0.05±0.01	0.06±0.00
Tumor	0.23±0.03	0.22±0.01	0.22±0.01	0.19±0.01
Tumor / Blood	0.31	0.58	0.81	0.29

*values are the average of five animals

The optimum formulation of the kit described above was found by testing several preparations without ascorbic acid, inositol or both. These formulations gave poor yields when reconstituted with pertechnetate.

The organ distribution data in mice of ^{99m}Tc-[V]-DMS are shown in Table 1. As expected the technetium tumor agent showed rapid blood clearance, low kidney uptake and significant bone concentration.

The distribution data of ^{99m}Tc-[V]-DMS in rats bearing walker sarcoma, presented in table 2, clearly demonstrate that tumor to blood ratio was increased progressively by time. Three hrs post administration a ratio of 0.81 was observed, while for ^{99m}Tc-DMS kidney agent tumor/blood ratio was found approx. three times less (0.29).

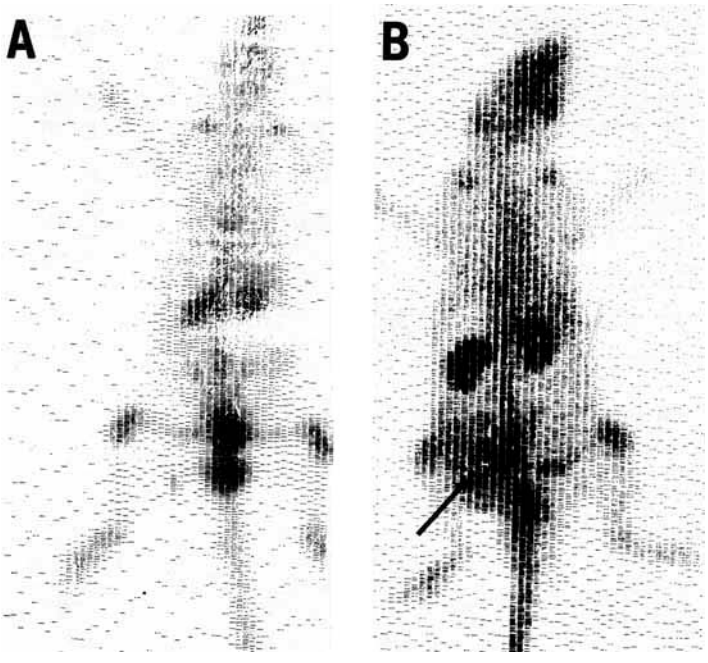

Fig. 3 Scintigrams of rats 2 hrs post administration of ^{99m}Tc-[V]-DMS. A: Healthy animals. B: Rats bearing walker sarcoma.

Scintigrams in rats 2 hrs post administration confirmed the concentration of ^{99m}Tc-[V]-DMS, prepared by the kit method, in the tumor (fig. 3). Further evaluation of the kit formulation was performed in human volunteers suffering from medullary thyroid

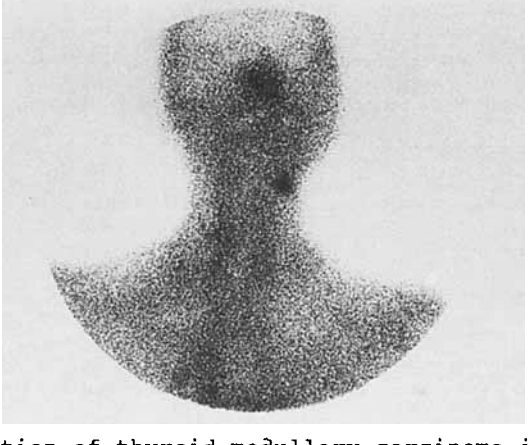

Fig. 4: Detection of thyroid medullary carcinoma in patient, 2 hrs post administration of ^{99m}Tc-[V]-DMS prepared by the kit method.

carcinoma. Tumor was clearly detected 2 hrs post administration of the radiopharmaceutical (fig. 4).

In conclusion the data obtained showed that the present formulation developed for the preparation of technetium DMS tumor agent gave a good quality radiopharmaceutical. Reconstitution of the kit and labeling with Tc-99m is performed in a single step. ^{99m}Tc-[V]-DMS was found stable six hrs after preparation.

Radiochemical control methods presented, permit a rapid and accurate determination of the radioactive impurities in the radiopharmaceutical preparation.

The kit showed a prolonged stability when it was stored in the freezer. Analytical control for a six month period revealed over a 95% formation of ^{99m}Tc-[V]-DMS complex showing the desired biological behavior.

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