AJPS, 2009, Vol. 6, No.1

Preparation and Clinical Evaluation of ^{99m}Tc-dimercapto Succinic Acid (^{99m}Tc-DMSA), Freeze-dried Kit For Kidney Cortex Scintigraphy

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الخلاصة

حضرت عدة طبية جاهزة من حامض الداي ميركبتوسكسنيك معلمة بالتكنيشيوم−99م. تحتوي العدة على 1 ملي غرام حامض 3,2 داي ميركبتوسكسنيك, 0.336 ملي غرام كلوريد القصديروز, 40 ملي عرام أنيستول, و 0.9 ملي غرام حامض الاسكوربيك مضاد للأكسدة وبدالة حامضية تتراوح (2−4) في ظروف مثالية لتعليم العدة بالتكنيشيوم−99م وتكوين المعقد.

قيست النقاوة الراديوكيمياوية واستقرارية العدة في الحالتين السائلة والجافة باستخدام تقنية كروماتوغرافيا العمود ذات الطور الثابت الصلب "الهلام" من مادة السيفادكس −G25.

أشارت نتائج التحاليل الراديوكيمياوية أن النقاوة الراديوكيمياوية للعدة المعلمة بالتكنيشيوم−99م أكثر من 99% بعد 15 دقيقة من زمن التعليم ومستقرة في حالتها السائلة لمدة عشر ساعات أما في حلتها الجافة مستقرة لمدة ستة أشهر.

درس التوزيع البايولوجي للعدة على ذكور الفئران البيضاء حيث كانت نسبة التركيز للجرعة المزروقة في الكلى قد بلغت 29% أما في الاعضاء الغير مرغوبة فكانت نسب قليلة جدا.

قيمت العدة سريريا باستخدام جهار الكاما كاميرا وعلى ألمتطوعين وأعطت نتائج ممتازة عندما قورنت بالنتائج السريرية للعدة المستوردة.

Abstract

^{99m}Tc-dimercapto Succinic acid freeze-dried kit(^{99m}Tc-DMSA) was prepared by reaction of kit that is contained (1mg of 2.3 meso dimercaptosuccinic acid, 0.336 mg of stannous chloride, 40mg insitol and 0.9 ascorbic acid as anti oxidant) with $Na^{99m}TcO_4$ at pH equal to (2-4) that is the optimal condition to formation ^{99m}Tc-DMSA complex.

The radiochemical purity and stability of ^{99m}Tc-DMSA in liquid and dried forms were determined on gel filtration column scanning “GCS”. The labeling

yield of complex formation was greater than 99% after 15 min. from addition of $Na^{99m}TcO_4$. The complex in liquid form was stable during ten hours while in lyophilized form six months.

The biodisribution of kit was studied on white male mice that the results indicate to the ratio of dose concentration accumulated into kidneys is greater than 29% of injected dose, however, little amounts of complex was detected in non target organs.

Introduction

^{99m}Tc-DMSA scintigraphy is the most sensitive method to detect a renal defect and this agent concentrated in cortex principally in the proximal convoluted tubules for sufficiently long time to enable detailed scintigraphy evaluation [1, 2, 3]. Approximately 90% of ^{99m}Tc-DMSA is bound to the plasma proteins with limits glomerular filtration [4, 5].

^{99m}Tc-DMSA as a static renal agent in detection of renal scarring and allows accurate calculation of differential renal function "DRF" [6].

Piepsz, has reported that the main tool of radionuclide techniques applied to pediatric uro-nephrology is the quantitation of function which is an information not easily obtained by other diagnostics modalities.

^{99m}Tc-DMSA scintigraphy is an accurate method for evaluation of regional cortical impairment during acute pyelonegphritis and later on, for detection of permanent scarring [7].

Whiter et al, have reported that ^{99m}Tc-DMSA scintigraphy was significantly more sensitive than intravenous uro-graphy and altera sonography in the detection of renal parenchymal disease [8]. The main advantages of this agent are its slightly higher radiation dose in comparison with other renal agents because of tubular fixation of DMSA and time consumption which may require a delay of 24 hours [9, 10, and 11].

Bair et al, have reported that ^{99m}Tc-DMSA was more accurate in renal parenchymal lesions and preferable for the evaluation of differentional renal function "DRF" in patients who had abnormal position of the kidneys [12].

The relative uptake of ^{99m}Tc-DMSA corrected for renal size is a more accurate method for assessing individual renal function. When there is a small kidney, relative ^{99m}Tc-DMSA uptake corrected for renal size can be distinguished between a normal and a hypo functioning kidney [13].

In clinical practice, ^{99m}Tc-DMSA is an excellent renal parenchymal imaging agent that 30% of injected dose was located in the kidneys at one hour after injection [4].

^{99m}Tc-DMSA scintigraphy remains the gold standard method because of the major advantages of this method such as the availability of different acquisition projections and better delineation of the kidneys [6].

The aim of this work was to prepare ^{99m}Tc-DMSA lyophilized instant kit for cortex kidneys scintigraphy and applied on animals then on human to confirm the application results as clinical evaluation of kit.

Materials and Methods

All chemicals used in this work were the highest analytical grade obtained from commercial sources and without any further purification. Sterilized and deionized distilled water was used for all preparation. The chemicals were obtained from commercial companies; meso 2,3 dimercaptosuccinic acid was obtained from (SIGMA ,Chemicals (USA)) , Stannous chloride dihydrate (RIDEL SEEZE HANOVER (GERMANY)),

Inositol from (CARLORBA Milan ,(ITALY)) and L-ascorbic acid from (BDH, Chemical Co England). $Na^{99m}TcO_4$ was eluted from the ^{99m}Tc-generator obtained from (Amersham international PLC,England) ,Sephadex G-25 was obtained from (pharmacia AB Fine ,Chemical, Sweeden).

Preparation of lyophilized kit:

The preparation of kit was applied under aseptic condition. 50mg of meso 2,3 dimercaptosuccinic acid was dissolved in 5ml of 0.1N NaOH . 2grams of Inositol was dissolved in to 94ml of sterile, pyrogen free distilled water. DMSA solution was added into the inositol solution and mixed well then added 2ml of stannous chloride dropwise with continuous slightly shaking. 45mg of ascorbic acid was added then after the pH was (2.0-4.0). The DMSA preparation was passed through 0.2µm of Millipore filter membrane then dispended into 50 sterile vials "2ml per each vial". The contents of the vials were lyophilized and stored at 5 ^{o}C.

Radiochemical analysis:

The radiochemical purity was determined by using of "GCS" technique. A glass column with an inner diameter 15mm and 30cm length was filled to height with sephadex-G25 fine in saline. The sample was analyzed with 15ml of saline. The column was scanned with slit (1mm) collimated NaI (TL) crystal.

Organ distribution:

The organ distribution of ^{99m}Tc-DMSA kit was performed in white male mice (Balb/c) aged about 6 weeks weighting (25-30 gr). Aliquots of 0.1ml of kit in different form lyophilized and non lyophilized containing (10-25µci) were injected into tail veins of ten mice. The mice were killed using diethyl ether at different time intervals (half, one, two, four and six hours). The radioactivity in various organs was measured using well type scintillation counter (Gamma Zint BF 5300, Berthod, FRD)

Blood clearance:

The disappearance of ^{99m}Tc-DMSA preparation from the blood was determined in rabbits white Newzland type weighting (2.0-3.0kg) by intravenous injection of 0.1ml containing (500µci) through the marginal air vein. Multiple blood samples were withdrawn from the heart by intracardial puncture using heparin zed syringes at different time intervals. The samples were counted in a comparison with appropriate a standard using the same counting system.

Results and discussion

The radiochemical purity was applied to determine the percent of fractions that are found in the preparation, $Na^{99m}TcO_4$, ^{99m}Tc-DMSA and reduced hydrolyzed technetium form. Both fractions of ^{99m}Tc-DMSA and reduced hydrolyzed technetium form remaining at the top of the column while the free $Na^{99m}TcO_4$ migrated 6cm from the top of the column (fig.-1).

The formation of ^{99m}Tc-DMSA complex was pH dependent. The results in table-1 indicate that the percents of ^{99m}Tc-DMSA and $Na^{99m}TcO_4$ in the preparation as a function of pH using "GCS" technique. The highest radiochemical purity of ^{99m}Tc-DMSA was greater than 99% at pH values equal to 3.0.

The effect of $SnCl_2.H_2O$ concentration on the formation of ^{99m}Tc-DMSA complex is presented in table-2. The results indicate that the highest radiochemical purity was at the optimal ratio of DMSA: stannous ion 3:1.

The stability of the preparation in liquid form was shown in table-3. The results indicate that the preparation was stable for ten hours after addition of $Na^{99m}TcO_4$. The results in table-4 indicate that the optimal time reaction of Sn-DMSA with $Na^{99m}TcO_4$ was 15 minutes. The shelf life of the lyophilized form of Sn-DMSA kit was followed for six months. The radiochemical purity and kidneys uptake were high during this period as shown in table-5.

The organ distribution of ^{99m}Tc-DMSA was demonstrated in table-6, the kidneys uptake was 29% with low liver uptake 2.3% after six hours of injected dose.

The blood clearance curve of ^{99m}Tc-DMSA complex obtained in rabbits was shown in (fig.-2). The plotting curve resolved into two exponents, the fast and the slow components are three minutes and 240 minute respectively. These results were evident that the fraction is cleared from the circulation after 240 minutes which enable the physicians to get a clear imaging with gamma camera.

^{99m}Tc-DMSA was clinically diagnosis on human subjects. The contents of lyophilized kit were allowed to react with $Na^{99m}TcO_4$ to get radioactive concentration about 12 mci/ml. 0.5 ml was injected and a very good quality scintigrams of the kidneys were obtained in the 240 minutes post injection using a gamma camera (fig.-3) .The results were demonstrated the preparation and medical

application of ^{99m}Tc-DMSA for kidneys cortex scintigraphy. ^{99m}Tc-DMSA scintigraphy remains the gold standard method because of its well known advantages [6].

pH Values*	^{99m}Tc-DMSA(%)	$Na^{99m}TcO_4$(%)
2.0	98.00	1.40
2.4	98.50	1.44
2.6	98.00	1.56
2.8	99.40	0.70
3.0	99.40	0.50
3.6	99.40	0.60
4.0	99.20	0.55
5.0	50.20	49.60
5.5	48.30	51.76
6.5	43.30	56.70
7.0	30.00	70.00
8.0	21.10	78.99
9.0	20.0	80.00

* Mean of 5 experiments for each pH values.

Table 1: The radiochemical purity of ^{99m}Tc-DMSA Complex as a function of pH values.

W_1(mg)	W_2(mg)	W_1/W_2	^{99m}Tc-DMSA (%)	$Na^{99m}TcO_4$ (%)
1.0	1.0	1.0	90.42	9.58
1.0	0.66	1.5	99.00	1.00
1.0	0.5	2.0	92.90	7.10
1.0	0.33	3.0	99.30	0.70

Table 2: The effect of weight ratio of dimercapto Succinic acid (W_1) to stannous chloride (W_2) on the chemical purity of ^{99m}Tc-DMSA obtained by "GCS" technique using "sephadex G-25 fine" at pH=3.

Time after labeling (hours)	^{99m}Tc-DMSA* (%)	$Na^{99m}TcO_4$ (%)
1.0	99.10	0.90
2.0	99.00	1.00
3.0	98.90	1.01
4.0	99.00	1.00
5.0	99.30	0.70
6.0	99.50	0.50

*Mean of five experiments for each time.

Table 3: The stability of Sn-DMSA in (liquid form) Using "GCS" technique after 15 minute from adding of $Na^{99m}TcO_4$.

Labeling time (min.)	^{99m}Tc-DMSA (%)	$Na^{99m}TcO_4$ (%)
15	99.14	0.86
60	99.20	0.80
120	99.50	0.50
180	98.90	1.10
240	98.50	1.50
300	98.90	1.10
360	99.00	1.00

Table 4: The stability of ^{99m}Tc-DMSA using "GCS" technique

Time after preparation (Days)	^{99m}Tc-DMSA (%)	% **Dose / organs			
		Kidneys	Liver	Urinary bladder	Spleen
2.0	99.00	29.00	2.30	0.60	0.26
60.0	99.00	27.50	2.50	0.70	0.17
120.0	98.50	27.20	2.40	0.55	0.150
240.0	98.30	27.00	2.00	0.50	0.20
360.0	98.00	27.00	1.95	0.70	0.27

*Mean of five experiments for each time.

** Sacrificing time 240 minute.

Table 5: Determination of shelf life of Sn-DMSA freeze-dried kit with determination of radiochemical purity and organ distribution of ^{99m}Tc-DMSA in mice with labeling time 15 minute.

Time after injection (min.)	% Dose / organs			
	Kidneys	Liver	Ur-bladder	Spleen
60.0	29.50	2.60	0.90	0.21
120.0	28.90	2.30	0.99	0.35
180.0	28.00	2.70	1.53	0.41
240.0	27.10	2.00	2.90	0.65
300.0	27.00	3.00	3.00	0.710
360.0	27.00	1.90	3.40	0.77

Table 6: Organ distribution of ^{99m}Tc-DMSA in mice *.
* Mean of five mice in each time[8].

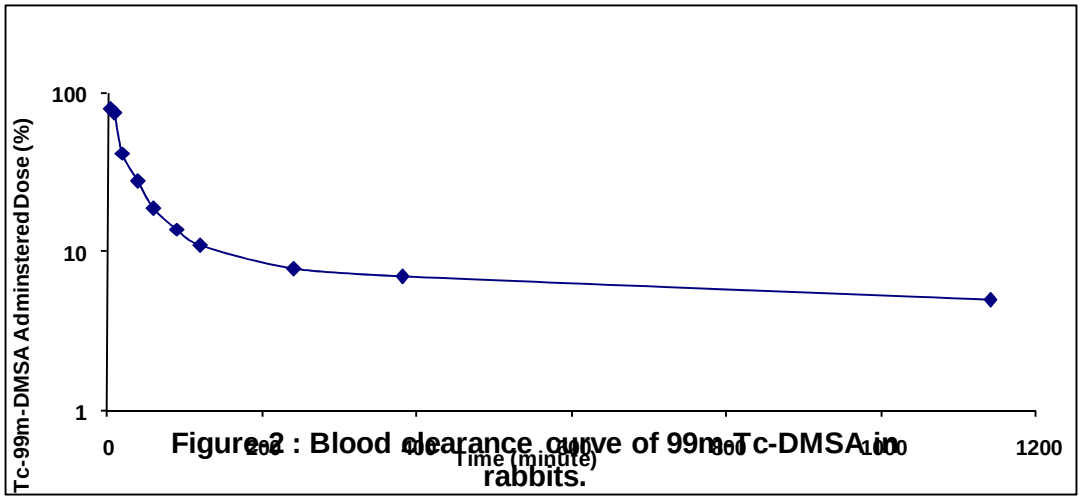
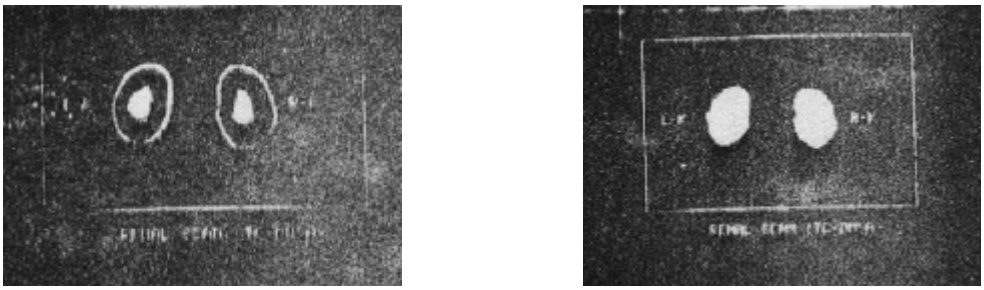


Figure 2 : Blood clearance curve of 99m-Tc-DMSA in rabbits.

* The results were the average of five rabbits.



a) Two hours post injection **b) Four hours post injection**

Figure 3: Normal kidneys scitigrams of ^{99m}Tc-DMSA was taken (a) two hours post injection and (b) four hours post injection.

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