

Preparation and evaluation of ^{99m}Tc -DMSA lyophilized kit for renal imaging

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Abstract: Dimercaptosuccinic acid (DMSA) has been evaluated and used with technetium 99m (^{99m}Tc) in imaging of kidneys. DMSA lyophilized kits were prepared and radiolabelled with ^{99m}Tc . Paper and thin-layer chromatography have been employed using various eluent systems for the radiochemical analysis, percentage labeling and binding capacity of ^{99m}Tc -DMSA. Female albino rabbits were used for this study. Biological data obtained after intravenous injection of radiolabelled DMSA to female albino rabbits revealed 32.42% uptake and long retention time in the kidneys. On the basis of animal biodistribution data, it is suggested that DMSA when labeled with ^{99m}Tc is useful complex for renal imaging and can be successfully applied as a diagnostic tool in nuclear medicine. Clinical biodistribution and radiation dosimetry studies are planned in future.

Keywords: ^{99m}Tc -DMSA, renal imaging, lyophilized kit, radiolabeling.

INTRODUCTION

To visualize any sort of abnormalities in the kidneys and any other organs of the body, different techniques are used. All radiological examinations begin with a plain radiograph before any contrast medium is injected and it is often very informative by itself in urinary surgery (Blendy, 1982). The intravenous urography is an imaging study of urinary tract morphology and not of renal function. This is achieved by the rapid intravenous administration of urografin or conray, a family of compounds based on benzoic acid attached to iodine atoms. Modern contrast media gives quite useful imaging even in the presence of significant impairment of renal function (Hussain, 1984). Ultrasound is another method of visualizing the internal structures of certain organs. When the history and earlier investigation leave uncertain the etiology of acute renal failure, then a percutaneous renal biopsy is indicated (Thompson and Woodhouse, 1987). But the risk associated with transrectal biopsy is urinary infection and bacteraemia (Eaton, 1981). Another method called Computed Tomography (CT Scan) is the non-invasive method of choice in the detection of supra renal tumors. It is very useful in locating retroperitoneal lesions, such as lymph node metastases, sarcomas and lymphomas, retroperitoneal fibrosis, abscess, urinoma, a non-functioning ectopic kidney or even abdominally retained undescended testis (Lee *et al.*, 1980). Wilms tumour (WT) with imaging is another useful and non invasive method in Europe (Smets and de Kraker, 2010; Brisse *et al.*, 2008). From the last two decades, imaging, a non-invasive technique has become very popular in the diagnosis of different abnormalities. Renal cell carcinoma (RCC) is the most common renal tumour in adults. It presents very rarely in childhood (1%) (Sausville *et al.*,

2009). Gamma camera scanning is the only non-invasive method of obtaining information regarding both, the morphological structure of the upper tract system and the functioning status of the renal unit. Wide range of radio isotopes are used for this purpose. Technetium-99m (^{99m}Tc) is the most widely used radionuclide in renal imaging. Renal imaging and functional studies are recognized as reliable means to evaluate the structure and location of the anatomic components of the urinary tract as well as to determine the total and differential performance of the kidneys. This can be done without danger of the allergic reaction, unpleasant side effects or excessive radiation dose to the organ involved. Complemented by other imaging modalities, radionuclide renal imaging has emerged as an important diagnostic tool in nephrology, urology and renal transplantation (Beschi *et al.*, 1981). Accumulation of radioactivity indicates the presence of functioning renal tissue and thus it is the technique of choice to search for ectopic renal tissue or to confirm the absence of a kidney (De Wardener, 1985). A large number of radioisotopes are available which can be used for gamma scintigraphy. In this study, ^{99m}Tc -Dimercaptosuccinic acid (^{99m}Tc -DMSA) has been used for scanning the renal tissues. 2,3-Dimercaptosuccinic acid was prepared by Owen and Sultanbawa (1949) and first used in the treatment of antimony, lead or mercury poisoning (Wang *et al.*, 1965). In 1974, ^{99m}Tc -DMSA was introduced as a substitute for radioactive mercury containing compounds for static imaging of the kidney (Lin *et al.*, 1974). ^{99m}Tc -DMSA has been used not only to visualize the kidneys to study renal morphology but also to assess individual kidney function. Despite its clinical use in assessing relative renal function, the mechanism by which ^{99m}Tc -DMSA is taken up by the kidneys is still not completely known. Two major probabilities are that it could enter the proximal tubular cell either by glomerular

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filtration and subsequent reabsorption or by direct uptake from the peritubular capillaries. Because, ^{99m}Tc -DMSA is largely bound to serum proteins 75%, according to Arnold *et al.* (1975) it is assumed that glomerular filtration is insignificant and that uptake takes place at the peritubular side of the cell. DMSA uptake is significantly decreased in the obstructed kidneys and uptake may improve following relief of obstruction (Taylor *et al.*, 1986). ^{99m}Tc -DMSA has a high sensitivity in the detection of a focal defect and allows estimation of differential function (Gordon *et al.*, 1992). ^{99m}Tc -DMSA imaging appears to be useful in detecting the distribution of amyloid deposits and in determining the appropriate site for biopsy and in detecting the tenosynovial giant-cell tumor and in diagnosing recurrence of this tumor (Kobayashi *et al.*, 1993). ^{99m}Tc -DMSA was used for the diagnosis of metastatic carcinoma of the prostate (Lamki and Shearer, 1984) and in the imaging of medullary thyroid carcinoma (Hilditch *et al.*, 1986). DMSA imaging is useful in patients with urethral duplication (Wu *et al.*, 1986) planar imaging (Pawana *et al.*, 1999) and for the evaluation of pyelonephritis and scars (Rushton and Majd, 1992; Gad *et al.*, 2004). Single photon emission computed tomography with ^{99m}Tc -DMSA (SPECT-DMSA) imaging is superior to ultrasound for the detection of renal scars and abnormalities in children with vesicoureteric reflux (Kibar *et al.*, 2003) and renal scintigraphy in children (Françoise *et al.*, 2003). Keeping all these points, ^{99m}Tc -DMSA has been selected for the renal imaging and for determining its rate of clearance from the renal region.

MATERIAL AND METHODS

Kit preparation and evaluation

We have prepared and evaluated the DMSA kit in our lab with the help of known literature methods. ^{99m}Tc labeled DMSA was first used by Lin (1974) and has been widely used for renal imaging. DMSA kits were prepared and evaluated by Vanlic and Gorkic (1976) and Vanlic and Petrovic (1982). Ikeda (1977) and Westera *et al.* (1985) reported four DMSA complexes, complex 1-4. The main differences between these complexes were change of pH and quantity of stannous chloride (SnCl_2). See Table 1.

Table 1: Four reported DMSA complexes

Complexes	Description	pH
Complex 1	DMSA+ SnCl_2 (less quantity) + $^{99m}\text{TcO}_4$	2.5
Complex 2	DMSA+ SnCl_2 (excess quantity) + $^{99m}\text{TcO}_4$	2.5
Complex 3	Complex 1 with pH 9.0	9.0
Complex 4	Complex 2 with pH 9.0	9.0

Among these complexes, the complex 2 showed highest kidney uptake. Formation of complex 2 from complex 1 greatly depends on ^{99m}Tc -concentrations and $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$

ratio. Change in pH is responsible for the formation of other complexes. Washburn *et al.* (1995) produced a pentavalent Tc-DMSA kit for medullary thyroid carcinoma and other tumors. We had investigated various ratios of DMSA and stannous chloride at variable pH values.

Medical experiments

Female albino rabbits were used for this study. The rabbits were taken from the well established and maintained animal house of INMOL and the kit was prepared in the Radiopharmaceutical laboratory of INMOL. The studies were carried out under gamma camera in the Hospital (INMOL).

Radiolabeling of kits

Radiolabeling of kits was simply done by reconstituting the kits with 2mL of $^{99m}\text{TcO}_4$ elute and incubating the kits for 10 minutes.

Evaluation of kits

Billinghurst (1973) developed good elect systems for paper and thin-layer chromatography for the analysis of radiopharmaceuticals. Several eluent systems have been developed for chromatographic analysis of ^{99m}Tc -DMSA by different researchers (Vanlic *et al.*, 1982; Westera, *et al.*, 1985) (table 2).

Table 2: Various solvent systems used for analysis of ^{99m}Tc -DMSA kits

S. No.	Mobile Phase	Com-position	Method	R _f Values
01	Acetone	-	P C/ TLC	A = 0.0 B = 1.0
02	Methanol : Water	(85:15 v/v)	P / TLC	A = 0.0 B = 0.9
03	Dioxane : Water	(9:1 v/v)	TLC	A = 0.0 B = 1.0
04	Ethanol : Water	(8:2 v/v)	TLC	A = 0.0 B = 0.9
05	n-Butanol: Acetic acid : Water	(3:2:3 v/v)	TLC	A = 0.0 B = 0.8
06	Chloroform: Methanol : Water	(80:40:3 v/v)	TLC	A = 0.0 B = 0.7
07	Ethanol : Water: Ammonia	(96:16:1 6 v/v)	TLC	A = 0.0 B = 0.8
08	Physiological saline	-	TLC	A = 0.0 B = 0.9

A = ^{99m}Tc -DMSA. (Complex),

B = Free pertechnetate ($^{99m}\text{TcO}_4$)

RESULTS

Kit preparation and radiolabeling

The ^{99m}Tc -DMSA Complex was prepared and evaluated

according to the methods of Billingham (1973) by paper and thin-layer chromatography. It was found that ^{99m}Tc -DMSA did not move along the solvent front and remained at the origin. 96 to 98% labeling of the ^{99m}Tc -DMSA was observed (table 3).

Table 3: Percent count in various solvent systems used for analysis of ^{99m}Tc -DMSA kits

S. No	Mobile Phase	% Count of ^{99m}Tc -DMSA. (Complex)	% Count of Free pertechnetate ($^{99m}\text{TcO}_4$)
01	Acetone	98	2
02	Methanol : Water	98	2
03	Dioxane : Water	97	3
04	Ethanol : Water	97	3
05	n-Butanol : Acetic acid : Water	98	2
06	Chloroform : Methanol : Water	98	2
07	Ethanol : Water : Ammonia	96	4
08	Physiological saline	98	2

Physical studies

Effect of pH on Labeling Efficiency

Maximum stability and labeling yield was obtained at pH 3-4.

Chemical studies

Paper Chromatography

Fig. 1 shows the determination of free pertechnetate in ^{99m}Tc -DMSA complex using Whatman No.1 paper in Acetone and 85% Methanol solvent system. ^{99m}Tc -DMSA remained stable in both cases ($R_f = 0$), whereas free pertechnetate migrated close to the solvent front ($R_f = 1.0$ and 0.9 respectively).

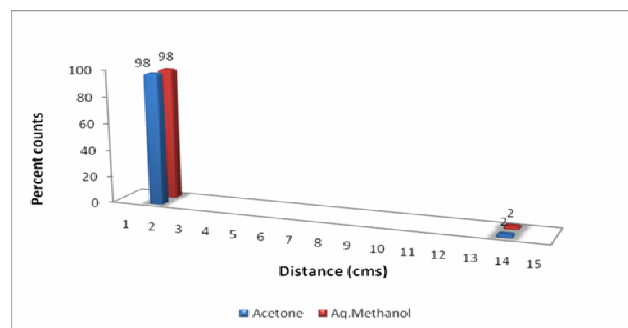


Fig 1: PC/TLC of Tc-DMSA on Whatman No.1 Paper/TLC plate in Acetone & aq. Methanol.

Thin-layer chromatography

Eight various solvents were used for assessing the stability of the ^{99m}Tc -DMSA complex on thin-layer of silica gel. ^{99m}Tc -DMSA complex was found at the origin ($R_f = 0$) in all solvent systems, whereas the free pertechnetate migrated close to the solvent front (fig. 2). Moreover, it was found that TLC of ^{99m}Tc -DMSA with the above solvent systems indicated that technetium was quantitatively reduced by the use of lower concentration of SnCl_2 , so the final product did not contain free pertechnetate.

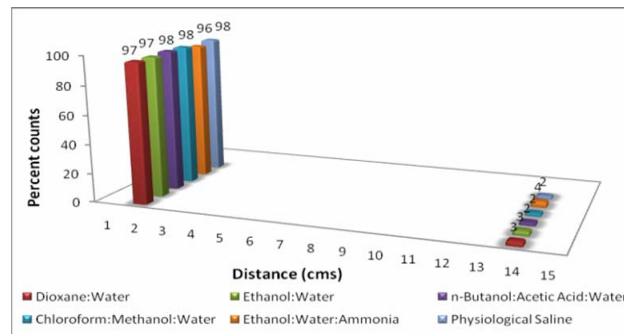


Fig. 2: TLC of Tc-DMSA on TLC plate in various solvent systems.

Stability studies

Stability of the ^{99m}Tc -DMSA depends largely on the temperature. Stability of the complex decreased as the temperature was raised. Although a slight decrease of ^{99m}Tc -DMSA was observed after 4 hours, the kit was stable for 24 hours when stored at a room temperature below 30°C . The shelf life extended to 7 months when stored at 25°C (fig. 3).

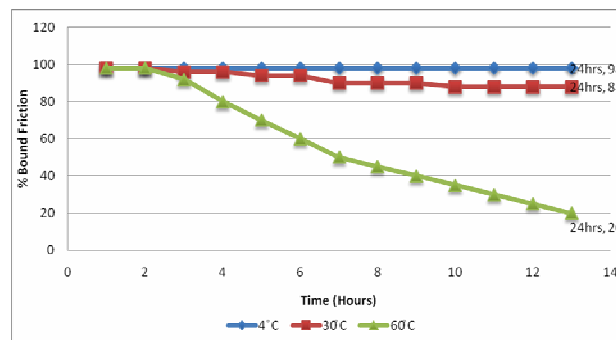


Fig 3: Stability of Tc-DMSA Kits stored at various temperatures.

Effect of concentration

The optimum concentration of the final solution used for the complex is 0.5 mg/mL for DMSA and 0.3 mg/mL for $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (each vial contained 2mL solution).

Effect of temperature

Evaluation at increased temperature resulted in deterioration of the complex.

Biological studies

Kidney imaging studies revealed that mean percent uptake of the injected dose of ^{99m}Tc -DMSA complex by the kidneys was $23.33 \pm 1.12\%$ one-hour post injection. Kidney uptake of ^{99m}Tc -DMSA was slow and it was found that the kidney uptake was high, 32.42% between third and fourth hour (fig. 4).

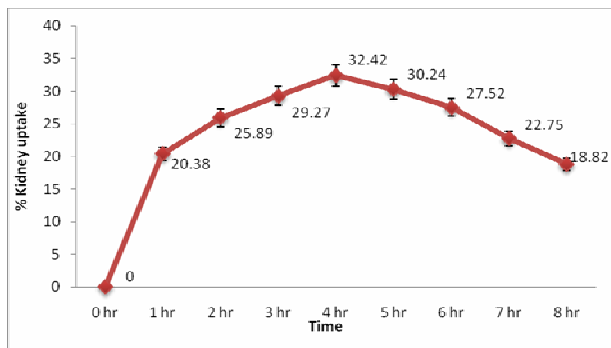


Fig. 4: Kidney uptake of ^{99m}Tc -DMSA.

DISCUSSION

^{99m}Tc -DMSA is largely bound to serum proteins 75%, according to Arnold *et al.* (1975). It is assumed that glomerular filtration is insignificant and that uptake takes place at the peritubular side of the cell. DMSA uptake is significantly decreased in the obstructed kidneys and uptake may improve following relief of obstruction. Gamma scintigraphic studies revealed that the mean percent uptake of the injected dose of ^{99m}Tc -DMSA complex by the kidneys was $20.38 \pm 1.12\%$ one-hour post injection. Kidney uptake of ^{99m}Tc -DMSA was slow and it was found that the kidney uptake was high, 32.42% between third and fourth hour (fig. 4). Some activity was picked up by the liver, but the successive scintigraphs of the kidney following injection of ^{99m}Tc -DMSA showed progressive accumulation of ^{99m}Tc in the kidneys at least up to 4 hours. This indicates that ^{99m}Tc -DMSA complex could be used clinically for the renal scintigraphy with excellent visualization. The residence of the complex prolonged to 24 hours.

Biological aspect of ^{99m}Tc -DMSA kit has already been examined (Arnold *et al.*, 1975) but this kit was for the first time prepared locally and evaluated for the chemical behavior, relation between chemical and biological characteristics and analytical procedures for quality control of ^{99m}Tc -DMSA kit. Imaging the kidney with ^{99m}Tc -DMSA is useful for the identification of focal renal pathology.

CONCLUSION

DMSA- ^{99m}Tc kit was resulted in excellent visualization in kidney imaging, and eliminated approximately completely in 24 hours. Moreover, the kit was found to be

stable for 7 months and can be used effectively within this period. ^{99m}Tc -DMSA when injected to rabbits did not show any adverse effects and thus can be investigated further to be used safely in human beings with a wide safety margin. We have planned clinical bio-distribution and radiation dosimetry studies in future.

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