Dos and Don’ts that are Issued through Radiolabeling Process of DMSA (Dimercaptosuccinic Acid) by $^{99m}$TcO$_4^-$ as $^{99m}$Tc-DMSA(III), the Gold Standard Radiopharmaceutical for Renal Cortical Scintigraphy

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Abstract

Objective: Since DMSA (Dimercaptosuccinic Acid) is an important and more sensitive kit in the realm of renal scintigraphy, in the course of this enormous study throughout five years, all quality control results from more than 500 $^{99m}$Tc-99m radiolabeled DMSA kits and scans were studied to improve radiolabeling method through optimization of all involving parameters.

Methods: Instant thin layer chromatography (ITLC) (support: ITLC-Silicic-acid (SA), solvent: n-Butanol Saturated with 0.3N HCl) were used to calculate %radiochemical purity ($%_{Tc-99m}$ DMSA). Data from ITLC and scans were investigated to obtain the optimized radiolabeling method so as to decrease background uptake particularly liver uptake.

Results: Data suggested that if radiolabeling is performed under optimized involving parameters (Generator used day 2, SA=(15-20 mCi)/ (1cc) radiochemical purity=99.5% (Al$_2$O$_3 <5$ ppm), generator’s pH=4-4.5), the $%_{Tc-99m}$ dimercaptosuccinic acid (DMSA) will be ≥ 90-95%. Moreover, more intricate scans with abnormal biodistribution of radiotracer developed the expanded view on $^{99m}$Tc-DMSA(III) renal scan impression with regard to diseases that can affect the $^{99m}$Tc-DMSA(III) biodistribution. During this investigation, it was concluded that almost all diseases that influence liver and spleen including fatty liver, mononucleosis and similar illnesses could change routine biodistribution of $^{99m}$Tc-DMSA(III).

Conclusion: Finally, we suggested an optimized radiolabeling method $^{99m}$Tc-DMSA(III) ≥ 90-95%) to reach a precious imaging as more as we can decrease uncertainties about interpretation of $^{99m}$Tc-99m DMSA scan considering patient’s background to provide an effective diagnosis.

Keywords: Radiolabeling; $^{99m}$Tc- DMSA; Renal scintigraphy; Renal pharmacicals

Introduction

Kidney as one of the vital organs participates in homeostasis of human body [1] including production of 1, 25-dihydroxy vitamin D [2], maintenance of pH [3] and electrolytes [4] balance, production of red blood cell [5] and control of blood pressure [6] as well as clearance. Many problems related to renal diseases could affect whole body activities by interrupting biological cycles in either direct or indirect way [7]. Therefore, well-timed diagnosis and efficient treatments play important role in patient’s promotion and contraction of long-termed side effects. There is a variety of diagnostic methods applied in this field [8]. Renal scintigraphy is one of the interesting diagnostic methods because of physiological survey, providing dynamic-static renal studies and modified radiation dose in relation to some classic radiological methods [9]. Three different issues (Glucomerular Filtration, Tubular Secretion and Cortical Function) are discussed in renal scintigraphy. Therefore, there are supposedly three different series of technetium radiopharmaceuticals with regard to purpose.

Renal cortical radiopharmaceuticals

Renal cortical scintigraphy is one of the important imaging which is performed to study renal morphology because of probability of scars in related patients. Several kinds of renal problems including UTI, pyelonephritis, reflux, etc. lead to cortical renal scar emerging [10,11]. High percentage of glomerulus and proximal convoluted tubules home in cortex, therefore, cortex’s deficiencies can hardly decrease renal function [12]. It should be mentioned that if scar is diagnosed as early as it emerges, there is high possibility of reversibility through efficient therapy. Therefore, with a well-timed and certain diagnosis, suffering patient can be promoted. Nuclear medicine provides a special method with renal cortical imaging via reasonable sensitivity and specificity [13]. The radiopharmaceutical used for this purpose should be retained in cortex without remarkable secretion supposedly. Two common positions in cortex, sulphydryl groups and some cytoplasmic proteins, are the objects to capture renal cortical radiopharmaceuticals [14], $^{99m}$Tc-DMSA(III), as a gold standard to renal cortical radiopharmaceutical, is most likely to maintain in cortex through cytoplasmic protein, approximately 50% of injected dose 2 hours after injection [14], $^{99m}$Tc-glucoheptonate (GHA) as uncommon cortical radioagent is eliminated by glomerulus in sum, with 10%-15% of injected dose captured in

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cortex [15]. Because of low percentage of cortex accumulation, it is not commonly used in this field.

Kinds of 99mTc- DMSA complex

Generally, two different types of 99mTc- DMSA complex, 99mTc-DMSA(III) and 99mTc-DMSA(V), had been reported with regard to radiolabeling condition. Due to spatial and geometrical differences, each complex specifically has its own biodistribution that caused two distinct clinical uses [16]. It was proved which complexes will be outstanding hardly depended on pH and ratio of DMSA: SnCl2.2H2O. At acidic pH 2.5 and excess amount of SnCl2.2H2O, the 99mTc- DMSA(III) will be reached that aggregates in renal cortex and prepares the renal cortical scintigraphy. In low concentration of SnCl2.2H2O and alkali pH 7.5-8, the 99mTc-DMSA(V) will be outstanding ones that could be absorbed in carcinoma deficiencies [17]. During a study formation of 99mTc-DMSA complexes in wide range of pH and DMSA: SnCl2.2H2O ratios were investigated in detail and also third form of 99mTc-DMSA with optimistic potential to clinical uses was reported [18].

Characteristics of 99mTc-DMSA(III) injection

Up to 3 hours after injection, 40-50% of injected 99mTc-DMSA(III) dose (adults: 80MBq children: 15MBq) [19] retained in renal cortex and about half of that is eliminated by tubules. Without any disruption in vivo and in vitro factors (it will be discussed), 2-3 hours after injection, there isn't any significant background uptake, so well-timed scintigraphy could be performed. Because of both renal aggregation/ elimination, kidney is considered as both target and critical organ.

Ingredients of DMSA cold kit

All kit ingredients are categorized into active and inactive moieties (Table 1) (Ingredients of DMSA cold kit). Active moiety is considered as DMSA which is able to bind with Tc-99m technetium as 99mTc-DMSA(III). Inactive moiety is all excipients and reductants. Inositol is common excipient used in DMSA kit.

Methodology Applied

Materials

Chromatography paper, Silicic-acid (SA), was obtained from Agilent technologies Part No. A120B12. Mixture of solvent (n-Butanol Saturated with 0.3N NaCl), was prepared following USP pharmacopeia1.

Equipment

Biodex Medical System, UPTAKE STAND 2” CTYSTAL, Model 187-220, No. 120397604. This system has been equipped with well counter.

Methods

There are two kinds of chromatography systems to assay Tc-99m DMSA kit quality (Table 2) (Various chromatography systems used for calculation of % 99mTc-DMSA(III)). In this study DMSA kit was labeled by (15-70) mCi of fresh 99mTc-sodium pertechnetate according to brochure instruction. 99mTc-sodium pertechnetate added kit was shaken for 2-10 minutes and then left in room temperature. After that, radiochemical purity was calculated by chromatography SYS (1). In this five-year study, all of the parameters involved in radiolabeling process were investigated to figure out how they can shift range of % 99mTc- DMSA(III). That should be noticed throughout this five-year study more than 500 kits were radiolabeled. Some of the important interests involved in radiolabeling process including vacuum quality of cold kit, dilution, specific activity, generator’s involving factors (used day, pH and aluminum oxide releasing) were investigated. To study of each independent parameter, we made an attempt to select kits that all had been radiolabeled in the same condition except under studied parameter.

Discussion

Through this study which took as long as five years, all data obtained from radiolabeled DMSA kit were gathered around all involving parameters that might have affected the quality of radiolabeled kit with regard to % 99mTc-DMSA(III). All variables including temperature, shaking time, specific radioactivity (99mTc- sodium pertechnetate mCi/ml) and all days that generator if it would be milked to use, were studied in this deliberation in detail. All results depend on SnCl2, as a common reductant in kit and structure of DMSA.

Temperature and shaking time studies

In same condition of all parameters except temperature and shaking time, results from 150 radiolabeled kits performed in range of room temperature (20-25) °C and shaking time (2-10) minutes, showed that there isn't a significant difference between obtained %radio labeling. It demonstrates that shaking time and mentioned temperature range don't have meaningful effect on radiolabeling process. It should be mentioned that if it is performed under incubation time less than 10 minutes without any shaking it will decrease %radio labeling.

Vacuum quality of cold kit

At first, it must be underlined vacuum DMSA kits had been used in this investigation (instead of nitrogen filled kits). All data for surveying this matter were fortuitously come from non-vacuum kits during study of other parameters (19 kits). It was concluded that the received data from non-vacuum cold kit were 15-20% (13% in average) decreased in comparison to ideal ones (Figure 1). It was estimated that interaction of
SnCl₂ and trapped air in the kit due to SnCl₂ oxidation to Sn⁺⁴ (Equation 1) that could obviously diminish the %radiolabeling because of usable Sn⁺² deficiency [20]. Furthermore, oxidized and hydrolyzed stannous as colloidal particles have a tendency to technetium-99m that will lead to liver aggregation of ⁹⁹ᵐTc-DMSA(III) [21]. Results suggest that non-vacuum kit should not be radiolabeled with regard to chromatography system SYS (1) that rules %radiolabeling should be more than %85. All non-vacuum Tc⁹⁹m-kits are not preferable but more decreased %radiolabeling on DMSA estimating interference of ⁹⁹ᵐTc-Sn colloid on ⁹⁹ᵐTc-DMSA(III) bio distribution.

6SnCl₂(aq) + O₂(g) + 2H₂O(l) → 2SnCl₄(aq) + 4Sn(OH)Cl(s)

(1)

**Dilution effects**

During investigation, it has been concluded that the amount of total volume (Vₜ) in radiolabeled kit could lead to change in clearance rate of ⁹⁹ᵐTc-DMSA(III) from blood pool and soft tissue. Almost 50 ⁹⁹ᵐTc-DMSA (III) injected patients, despite high-quality radiolabeled kit and acceptable range of serum BUN and creatinine, associated with more soft tissue retention in delayed imaging step (3 hour after injection) (Figure 2). Detailed studies proved that all ⁹⁹ᵐTc-DMSA (III) kits were diluted by normal saline nearly to Vₜ = 5-7 cc.

Results suggest that there is necessity, radiolabeled DMSA kit must be diluted up to 4 cc.

Vₜ = V (⁹⁹ᵐTcO₄⁻) + V (normal saline) (for dilution) ≤ 4cc

**Specific activity (SA)**

All gathered QC (quality control) results from nearly 500 kits that were radiolabeled with wide range of ⁹⁹ᵐTcO₄⁻ (15-70 mCi)/(1-4cc) during 5 years, fortuitously 80 radiolabeled kits performed in same condition except (SA), had been selected to investigate based on the ITLC results. This study suggested that in same conditions, the kits that were performed in ⁹⁹ᵐTcO₄⁻ (15-20 mCi)/(1cc) caused an increase in % radiolabeling %4 in average. Furthermore, we found that Tc⁹⁹m sodium pertechnetate added kits more than 60 mCi were accompanied by free Tc⁹⁹m sodium pertechnetate according to SYS (1).

**Radiochemical purity**

All generators must be controlled in terms of releasing alumina to perform in high radiochemical purity. The limited concentration of Al₂O₃ was reduced to 10 µg/ml in United States Pharmacopeia to prevent probability of Tc-99m Al₂O₃ formation [22]. Generator quality control test showed that cholorometry results in range of Al₂O₃<5 ppm causes radiochemical purity in range (99.5-99.69) % [23]. Assuming Al⁺³ (as lewis acid) affinity to DMSA (Dimercaptosuccinic Acid), it is causes radiochemical purity in range (99.5-99.69) % [23]. Assuming Al⁺³ (as lewis acid) affinity to DMSA (Dimercaptosuccinic Acid), it is causes radiochemical purity in range (99.5-99.69) % [23]. Assuming Al⁺³ (as lewis acid) affinity to DMSA (Dimercaptosuccinic Acid), it is causes radiochemical purity in range (99.5-99.69) % [23]. Assuming Al⁺³ (as lewis acid) affinity to DMSA (Dimercaptosuccinic Acid), it is causes radiochemical purity in range (99.5-99.69) % [23]. 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received day in order to probability of high concentrated radiolysis production, so it would be just undergone milking and drying without contribution in radiolabeling process. All 100 kits had been labeled on different days from Day 2 to Day 8 (latest used day), in same condition of batch number, specific activity, etc. results showed that the kits radiolabeled on Day 2, raise % of -DMSA(III) up to 5% (in average) in comparison to other days. Minimum amounts of % have been recorded on Day 8 (Figure 4). It would be guessed that an increase in % along the week can slightly cause %radiolabeling to go down. It should be regarded all parameters that can increase colloid, decrease quality of kits, but could affect DMSA more than others in order to DMSA structure. Two critical days after generator production (2nd, 8th), were more focused in this investigation.

**Other Factors That Could Affect Kit Quality or Change Routine Biodistribution of 99mTc - DMSA(III)**

**The amount of excipient in the cold kit**

During this study we rarely received scans associated with mild bone up take particularly in spinal column and hip (Figure 5). More investigations developed proof that related to imbalanced ratio of kit ingredients. Generally, the cold kit of DMSA is promoted by several excipients as productive agents. Inositol is a well-known agent of excipients ilk in this kit. It would mind that the ratio of Inositol/DMSA should be indeed managed because of competition on radiolabeling with 99m-Technetium (Figure 6). If the amount of inositol is more than optimized range, the bone uptake will be appear caused by tendency of radiolabeled inositol to hydroxyapatite's phosphate groups (Figure 6).

**External disrupting materials**

Recently, it was reported that if antiseptic added chlorhexidine...
is employed, it could significantly interfere in DMSA radiolabeling process. It backs to past that chlorhexidine accelerates colloid complex in technetium added DMSA kit [24].

**Some Diseases would Determine to Change 99mTc-DMSA(III) Biodistribution**

**Fatty liver**

Our studies demonstrated that in patients with high blood LDL, would cause fatty liver, the liver uptake of 99mTc-DMSA(III) will be considerable but the liver uptake race depends on fatty liver grade (Figure 7).

**High acidosis and alkaline condition**

According to mechanism of renal cortical retention which performed by cytoplasmic protein of cortical cell, high concentration of $H^+/OH^-$ in plasma disrupts $^{99m}$Tc-DMSA(III) cortical retention in order to OH$^-$/H$^-$ saturated proteins in charge for renal cortical retention of $^{99m}$Tc-DMSA(III). In the mentioned condition, in spite of the high-quality radiotracer, liver uptake will be considerable (Figure 8).

**Dehydration effects**

It was found that patients under dehydration lead to decreased uptake of $^{99m}$Tc-DMSA(III) because of decreasing capacity of kidney

**Acute renal failure (ARF)**

Generally, in ARF patients, all agents that should be interfered by kidney tend towards liver because of renal function deficiencies in comparison to normal cases. In these patients, liver will be basically appeared however low cortical retention of $^{99m}$Tc-DMS1A (Figure 9). Results showed that there is a correspondence between creatinine levels and kidney uptake. The high ranges of creatinine will lead to more liver uptake.

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**Mononucleosis infection**

Through our investigation, as an interesting result it was proved that patient bearing mononucleosis must shift biodistribution of $^{99m}$Tc-DMSA(III) toward liver and spleen. $^{99m}$Tc-DMSA(III) scintigraphy of mentioned patients showed that scan was accompanied with high aggregation of $^{99m}$Tc-DMSA(III) in reticuloendothelial organs despite high quality of radiopharmaceutical, there isn’t just kidney retention (Figure 10).
4.5, diluted radiolabeled kit up to 4cc, raise percentage of % optimized of each parameter, the more raising % radiolabeling. It approaches to raise % radiolabeling. The more performing under cortical scintigraphy by high-quality radiopharmaceutical as well as considering patient’s background prepares a precise scan interpretation that leads to accurate diagnosis and patient promotion.

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