RADIOPHARMACEUTICAL FOR IMAGING OF LIVER, GALL BLADDER AND BILIARY DUCTS(^{99m}TC-DISIDA)

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ABSTRACT

The cholescintigraphic procedures have played a significant role in diagnosis of hepatobiliary disorder in Thailand. Especially ^{99m}Tc-DISIDA scintigrams of infants are the diagnosis procedures of choice for biliary atresia because it is difficult to diagnose by other means for examples CT and ultrasonography. The synthesis of DISIDA (2,6-diisopropylacetanilide iminodiacetic acid), was prepared successfully since 1983 at Section of Nuclear Medicine, Siriraj Hospital. The DISIDA compound was made into DISIDA instant kits by adding appropriate amount of stannous chloride. Each kit contained 10 mg of DISIDA and 0.25 mg of $SnCl_2.2H_2O$. Radiochemical purity was good at 2-4 ml of Na $^{99m}TcO_4$ (activity of 1 to 50 mCi) and lasted for 6 hours. The Lyophilized form of DISIDA-kit had stability not less than 1 year. No reports of unfavorable effects on patients with this product during the past 14 years.

INTRODUCTION

It is unfavourable to use ^{131}I compound for liver diagnosis especially with scinticamera imaging because of its high gamma energy . The absorbed radiation dose of ^{131}I is too high when comparing with ^{99m}Tc. ^{99m}Tc is one of the ideal radionuclides for the diagnostic purpose. It is carrier free and its isotopic abundance is 100%. The chemical mass is negligible and the binding sites of such compound as DISIDA (2,6-diisopropylacetanilide iminodiacetic acid) should be able to bind relatively large quantities of ^{99m}Tc. This radionuclide also decay to give a high photon yield of good energy (90%,140 KeV). The advantages of this radiopharmaceutical include its safety and accuracy with low radiation dose[1] and decreased dependence on hepatic function. A unique feature is their ability to depict the physiology and dynamics of biliary excretion.[2,3,4]

MATERIALS AND METHODS

Analytical grade or equivalent reagent chemicals, water, sterilized and pyrogen free, as well as sterile disposible syringes were used in all procedures. Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from DAINATEC generator from Japan. Lyophilization was achieved in a Hetosicc Freeze dryer type CD-13.2. The preparation was carried out in a Nuaire larminar flow biological safety cabinet. The electrophoretic mobility studies were carried out using Whatman No.1 paper strips (1 inch wide) in veronal buffer, pH=8.6, μ=0.075M, at a potential 200 volts for 4 hours in 5^0C surroundings.[5,6] The paper were placed under gamma camera. The radiochemical purity was determined by selecting areas of interest regions by the computer. The animal experiments were conducted in adult rabbits.

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1. Preparation of *a* chloro-2',6'diisopropylacetanilide

31.7 ml of 2,6-diisopropylanilide was dissolved in 150 ml of acetone and cooled in ice-bath. 21 ml of chloroacetyl chloride was added dropwise to the amine solution and allowed to stand for one hour. The solution was then poured into 200 ml of 10% HCl. The precipitate was collected by using Buchner filter set and washed with 0.01% HCl. Recrystallization was performed by dissolving the precipitate with 500 ml of 99.9% ethanol and 200 ml of distilled water was slowly added then the *a* chloro-2',6'diisopropylacetanilide was collected. Recrystallization was done again by using the same method until the *a* chloro-2',6' diisopropylacetanilide was very pure.[7]

2. Preparation of 2,6-diisopropylacetanilide iminodiacetic acid (DISIDA)

10 g of iminodiacetic acid was dissolved in 80 ml of distilled water, 5 g of *a* chloro-2',6' diisopropylacetanilide was dissolved in 100 ml ethanol. Under 80°C reflux, the latter was slowly dropped from the separating funnel to the former. After 20 hours the reaction was complete, ethanol was removed by distillation at 45°C for 4 hours. The residue was acidified with 37% HCl. The precipitate was collected on sintered filter and washed with distilled water. The product was purified 3 times by recrystallization using the above method. Finally the precipitate was dissolved in 300 ml of ethanol and 150 ml of water and allowed to crystallize in the freezer. The product was collected and washed with cold ethanol, dried and stored.[8,9]

3. Preparation of DISIDA kits

1 g of DISIDA was suspended in 50 ml of distilled water for injection and dissolution was effected by the gradual addition of about 0.4 ml of 40% NaOH while stirring to a pH of 6 to 6.5. This solution was called solution A. 1 g of $SnCl_2.2H_2O$ was dissolved in 5 ml of concentrated HCl. This solution was stirred at least 30 min. and 15 ml of water for injection was added and called solution B. 0.5 ml of solution B was added to solution A. Then it was mixed well and readjusted pH to 6 with 0.1 ml of 40%NaOH. The solution was sterilized by passing through 0.22μm membrane filter and transfered 0.5 ml each to 7 ml presterilized serum vials. The content of each of the vials were lyophilized and closed with rubber closures. The process of closing the rubber was done in vacuum by using autometic stoppering arrangement. Finally, the vials with rubber closures were sealed with aluminium caps and stored at 0-5°C. Each kit contained 10 mg of DISIDA and 0.25 mg of $SnCl_2.2H_2O$.[10]

4. Lebelling DISIDA with ^{99m}Tc

2-4 ml of $Na^{99m}TcO_4$ (activity of 1 to 50 mCi) was added to DISIDA kit and mixed well. Let stand at least 5 min.

5. Quality control of ^{99m}Tc-DISIDA

Each lot of DISIDA kits has to be tested for radiochemical purity, stability, apyrogenicity, sterility and specific activity. The procedure will be explained as follows.

Assay The electrophoretic mobility studies were carried out using Whatman No.1 paper strips (1 inch wide) in veronal buffer pH 8.6, μ = 0.075M, at a potential gradient of 200 Volts for 4 hours, at 5°C atmosphere. The strips of paper were placed under gamma camera. Immages were recorded and data were stored on computer. Areas of interest were selected for ^{99m}Tc-DISIDA, ^{99m}Tc-Sn-colloid, free $^{99m}TcO_4$ and the whole paper strip regions as shown in Fig.1. The radiochemical purity was determined by dividing the activity at ^{99m}Tc-DISIDA area by the activity of the whole paper strip. It was found that more than 95% of the total activity was associated with ^{99m}Tc-DISIDA zone and less than 5% as free $^{99m}TcO_4$ plus ^{99m}Tc-Sn-colloid in the preparation.

Stability Shelf-life of the radiopharmaceutical is one year. The results showed that the DISIDA kit had excellent properties both in vitro and in vivo tests. Bench-life of the preparation was 6 hours.

Apyrogenicity 2 mCi in 1 ml of ^{99m}Tc-DISIDA was injected to three healthy adult rabbits. The results showed that our DISIDA kits met the requirement for the absence of pyrogen.[11]

Sterility The first and the last vials were sent to the Department of Microbiology for sterility test, the results were negative.

Specific activity Our kits could be mixed with 2 to 4 ml of 1-50 mCi of Na $^{99m}TcO_4$.

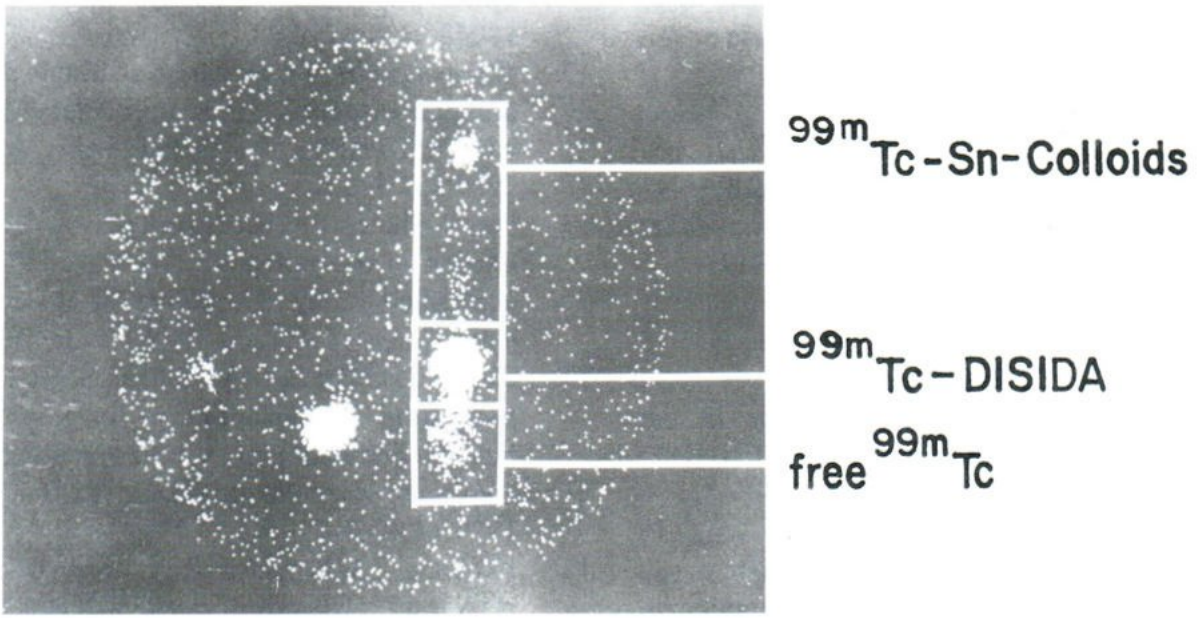

Fig. 1. Areas of interest were selected for ^{99m}Tc-DISIDA, ^{99m}Tc-Sn-colloid and free pertechnetate.

RESULTS AND DISCUSSION

a chloro-2',6'diisopropylacetanilide was synthesized from 2,6-diisopropylanilide and chloroacetylchloride. DISIDA (2',6'diisopropylacetanilide iminodiacetic acid) was synthesized from *a* chloro-2',6'diisopropylacetanilide and iminodiacetic acid. The DISIDA was made as DISIDA instant kit by addition of an appropriate amount of stannous chloride and stored in lyophilized form. DISIDA instant kit was used routinely instead of ^{131}I BSP. Radiographic immaging procedures such as oral cholecystography served a very useful purpose, but several of them are invasive and involve a certain degree of risk from the administered contrast media as well as discomfort to the patient. Cholescintigraphy have proved to be the most sensitive method available for documenting cystic duct patency or obstruction[12-17] and had become the diagnostic procedure of choice for acute cholecystitis.[12,18] In addition it has proved useful in postoperative patients because of its ability to evaluate ductal obstruction as well as detect the presence of cystic duct remnants and biliary leakage. Diagnosis of these organic causes of postcholecystectomy syndrome can result in relief of symptoms via appropriate therapy. By the same token, exclusion of these disorders can permit internist or surgeon to search elsewhere for the patient's cause of distress.[4]

Generally surgeons have been less enthusiastic about the role of nuclear imaging in clinical situations. This has been because(1) several nuclear imaging procedures have a limited diagnostic accuracy.(2) the scans generated often suffer from a lack of specificity and (3) the examinations have not been readily available and often had to be scheduled as special procedures. Despite these prior prejudices, their experiences with iminodiacetic (IDA) imaging of the biliary tract are changing their attitude.

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