

PREPARATION OF Tc^{99m}-Sn-THIOGLYCOLIC ACID-di-ISOLEUCINE (TC-99m-TGA-ILEU) COMPLEX AND ITS COMPARISON WITH TI 201- CHLORIDE FOR MYOCARDIAL IMAGING

Pages with reference to book, From 40 To 43

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Abstract

DI-Isoleucine was complexed with Thioglycolic acid in the presence of Stannous ions to label with TC99m-Pertechnetate. Percentage labeling was determined by paper chromatography. Preparations of more than 95% labeled with Tc99m were injected to rabbits for imaging and tissue distribution studies. Tc99m.TGA.I LEU complex was investigated as a myocardial imaging agent with respect to its kinetics and its percent organ uptake. As compared with TI 201, Tc99m. TGA-ILEU complex shows higher heart to lung ratio and higher liver uptake. At the time of peak myocardial uptake, the mean heart uptake of TI 201 was 3.9% in rabbit compared with 3.3% for Tc99m.TGA. I LEU. At 2 hour post-injection TI 201 was found 1.9% and Tc99m.TGA.ILEU 1.5%. The kinetics of TC99m.TGA.ILEU complex warrant its further investigation, as a myocardial imaging agent (JPMA36:40,1998).

INTRODUCTION

Tc-99m-Pyrophosphate¹, Thallium-201^{1,2} and K-43, Cs-129, Rb-81^{4,5}, are being used in cardiac radionuclide studies. Tc-99m Pyrophosphate is used for positive infarct scintigraphy in early phase, while Thallium-201 is taken up by the viable myocardium and infarction is shown by negative scintigraphy. The uptake of Thallium-201 is proportional to the distribution of regional blood flow and has been utilised most widely. Functional tomographic images of myocardial metabolism and blood flow can also be obtained by using positron emitters, F-18-Glucose⁶ and N-13 Ammonia⁷. A Tc-99m(III) bis-(1,2-dimethyl-phosphino) ethane (DMPE) was designed for myocardial uptake and its kinetics have been studied in dogs⁸. Its biodistribution are quite encouraging and can be used as guide lines for further exploration of myocardium imaging agents. 1-13 1-Labeled fatty acids have been investigated for cardiac studies extensively⁹, but no significant achievement obtained.

Several Chelates of amino acids were prepared, by labeling with Tc-99m through Thioglycolic acid and studied for biodistribution in rabbits. Isoleucine complexed with Tc-99m was found to be taken by heart muscles, and its kinetics were compared with TI 201 Chloride.

MATERIAL AND METHODS

Preparation of the Complex Various concentrations of di-Isoleucine (BDH), Thioglycolic Acid (E.MERCK) and Sn++(BDH) were used to complex with Tc99m. Pertechnetate (Amersham International Limited).

The following procedure was found optimum. Dissolve 50 mgs of dl-Isoleucine in 100 ml distilled

water (A).

Dissolve 0.2 ml of 80% Thioglycolic acid in 100ml distilled water ().

Prepare 1% Stannous Chloride dihydrate solution in 0.3 normal hydrochloric acid (C).

Mix 1.0 ml of solution A and 2.5 ml of solution B in a vial. Stir the mixture for half an hour, add 0.5 ml of solution C, stir well and adjust the pH to 6.5 with dilute sodium hydroxide solution (D).

Add (1-10) millicuries of Tc99m-Pertechnetate to 1 ml of solution D, after passing through 0.22 µm millipore filter.

Paper Chromatography

Tc-99m.Sn-TGA-ILEU complex formation was checked for any unbound Sodium Pertechnetate by applying paper chromatography. Strips of 2x20 cm size of Whatman No.1 filter paper were used. The prepared complex was spotted over the lower portion of the strip containing 100-200 µCi of the preparation. The strips were run in methanol/water system (85:15) upto 15 cm and cut into 1 cm section. Activity at various portions of the strip was calculated by counting in a well-type scintillation counter.

In Vivo Studies

Distribution studies of Tc99m-TGA-ILEU complex was carried out by external imaging of the whole animal by injecting 2 mCi of the complex to healthy rabbits and performing scintigraphic studies at 20, 60 and 90 minutes post injection. Quantitative uptake and kinetics of the complex were determined by sacrificing the rabbits and determining the uptake in bladder, kidney, lungs, liver and heart muscles, counting their activity under the gamma camera or well type scintillation counter. Samples of blood were also collected for finding the disappearance of the complex from the body. For comparative studies to determine basic kinetics and imaging data, 2 mCi of Thallium 201 (a commercial preparation of AMERSHAM International) was injected to rabbits. Imaging and quantitative studies were carried out as with the Tc99m-TGA-ILEU complex by taking scintiphotos and performing tissue distribution studies.

RESULTS AND DISCUSSION

The results of paper chromatography obtained, helped in designing an optimum preparation of Tc99m-TGA-ILEU. As shown in Figure 1,

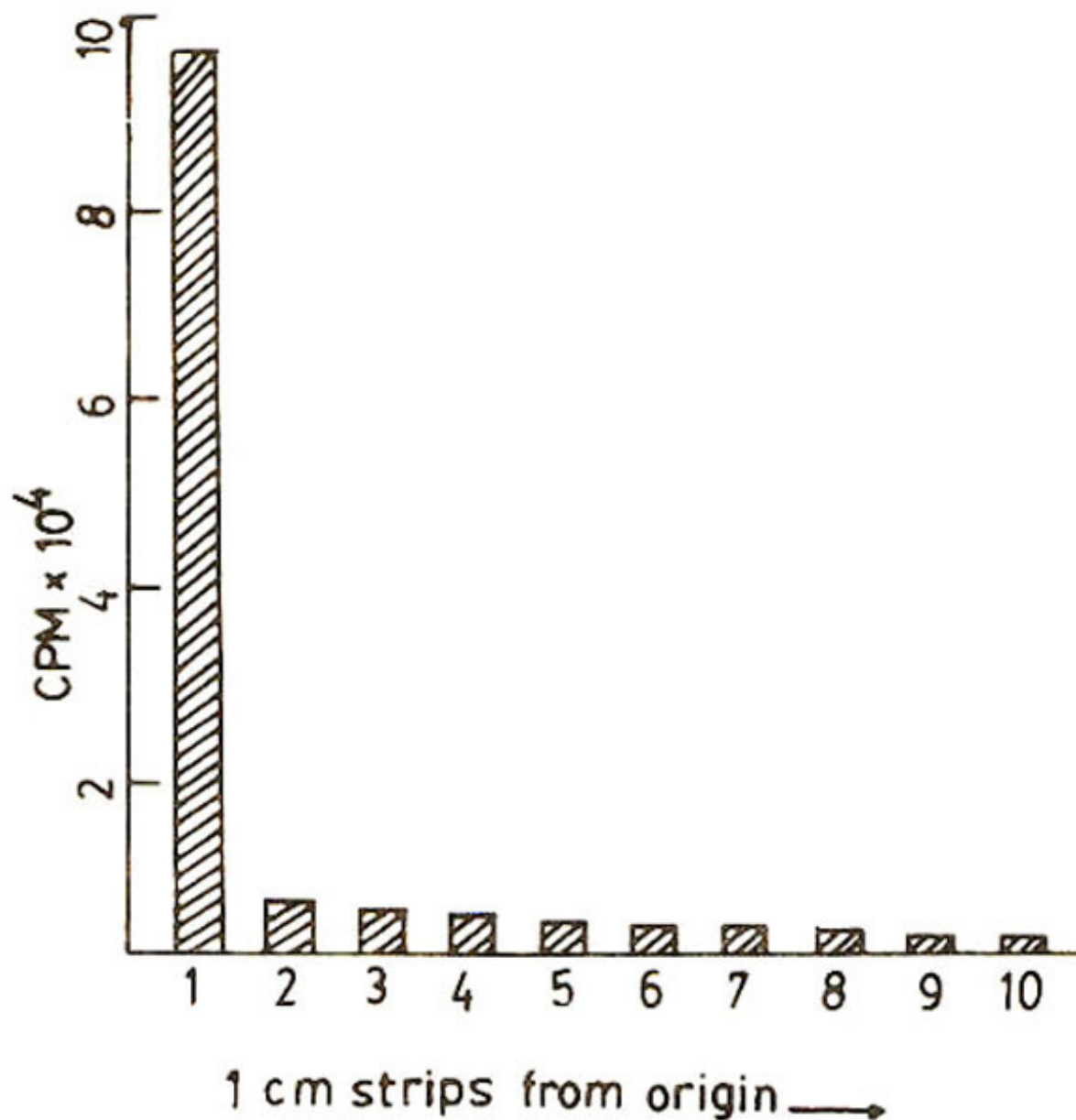


Figure 1. Percentage labeling of Tc-99m-Sn-TGA Isoleucine Complex.

no free pertechnetate was found. The preparation was stable for at least four hours. Over 95% labeling was achieved and the complex remained at the origin while the unbound pertechnetate if any moved along the solvent front at a R_F .6 to .8. This technique although does not ensure the distribution pattern of the complex but primarily it can guarantee the complexation. chromatography performed in 0.9% saline, showed negligible amount of hydrolyzed tc99m.

In Vivo Distribution

The in vivo distribution of the Tc-99m- TGA-ILEU complex in various organs of rabbits has been shown in Figure 2

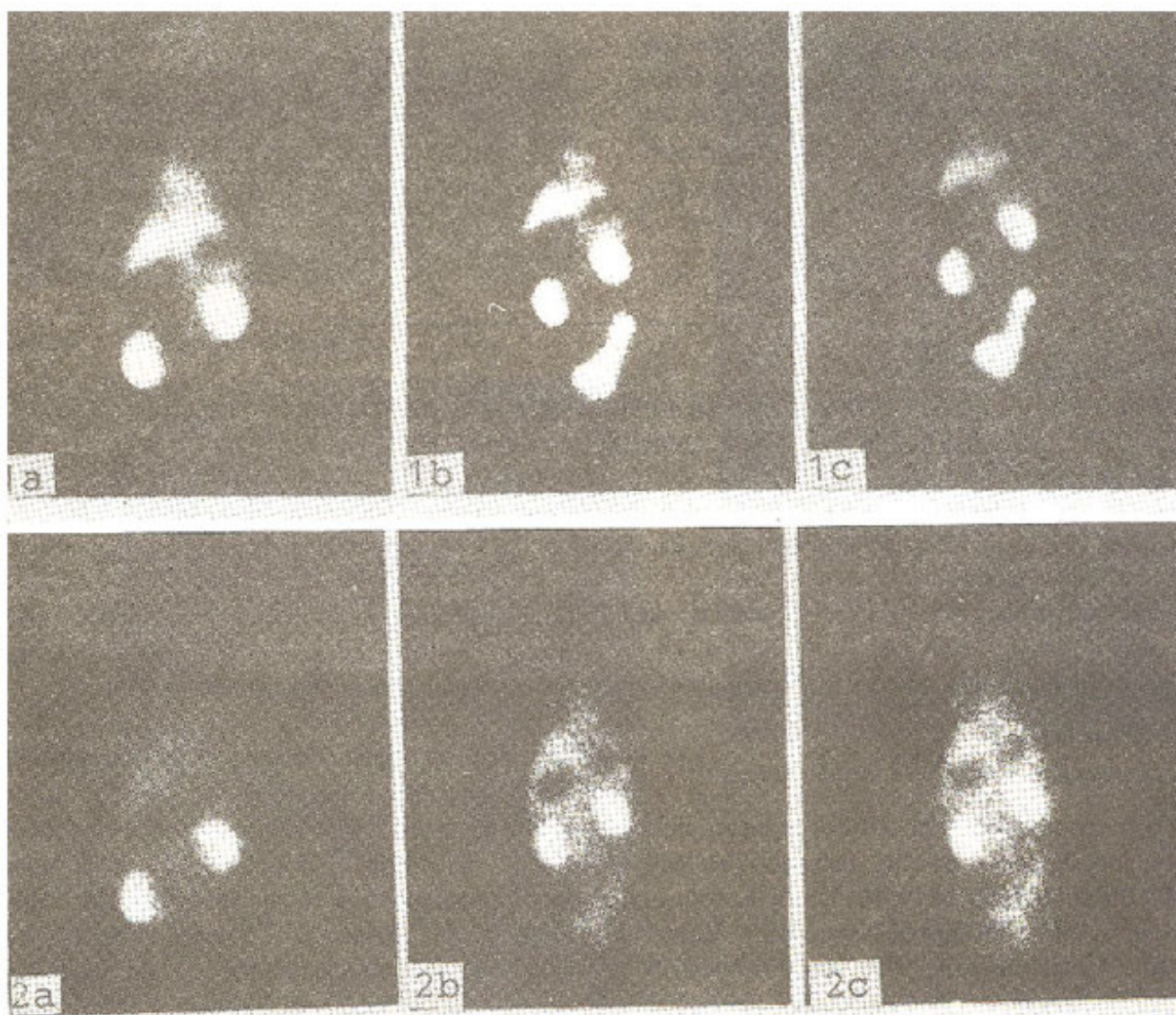


Figure 2. Images of the whole animal, (rabbits) at 20, 60 and 90 minutes post-injection.

i. Tc^{99m} -TGA-ILEU 1a, 1b, 1c. ii. TI 201 2a, 2b, 2c.

by taking scintiphotos at 20, 60 and 90 minutes post-injection under the-gamma camera, by collecting 300K counts. It appears that the complex excretes through the kidneys slowly but rapidly disappears from the blood compartment. Activity in the heart compared with the blood background. The diaphragmatic region of the heart is well-separated at 30 minutes post injection which is probably the optimum time for heart muscle imaging with Tc-99m-TGA-ILEU complex. Compared with images of TI 201, the pattern of distribution seems to be similar but the blood activity is higher in the case of TI 201, which disappears slowly from the blood as compared to Tc-99m-TGA-ILEU complex. TI 201 excretes slowly through the kidneys while in case of Tc-99m-TGA-ILEU complex bladder was visualized in the early phase.

Organ uptake in the case of Tc 99m-TGA-ILEU complex, are quite comparable with TI 201 as regards heart muscle uptake.

Table 1
Percent Organ Uptake in Rabbits of Tl 201 and
Tc^{99m}-TGA-ILEU Complex (n=5, Mean and Range).

Organ	Tl - 201	Tc-99m-TGA-ILEU
Heart	2.99(1.99-4.0)	2.4(1.5-3.3)
Lung	3.3(2.0-4.6)	0.9(0.7-1.1)
Liver	13.2(11.5-14.9)	15.3(13.4-17.3)
Kidney	11.3(6.9-15.8)	24.5(20.4-28.6)
Bladder	4.3(3.0-5.7)	26.9(23.2-30.6)

Table I shows the uptake of both the radionuclides in various organs of rabbit at 1 hour post-injection. The target organ takes up 3.9% of Tl 201 as peak uptake at 30 minutes post injection, while Tc^{99m} TGA-ILEU complex takes up 33% uptake at the same time of post-injection. The lung to liver ratio, which is very important in myocardial imaging, is favourable in the case of Tc^{99m}-TGA-ILEU complex, as the lung uptake of Tl 201 is quite high. Activity in the liver does not interfere with the heart imaging and is less than that of the Tc-99m DMPE complex⁸.

It has been attempted to compare the kinetics of Tc-99m-TGA-ILEU complex with Tl 201 in rabbits. Tl 201 has been widely accepted as the agent of choice for myocardial scintigraphy based on its myocardial distribution, which is closely proportional to regional myocardial blood flow. However its shortcomings include high cost and less than desirable physical properties as an imaging agent. Tc 99m being a radionuclide of choice possessing desirable physical properties has potential advantage. The kinetics of Tc-99m-TGA-ILEU complex are qualitatively similar to those of Tl 201 although the mechanism of its concentration in the myocardium is not known. Tc 99m-TGA-ILEU complex is a new agent whose kinetics under variable conditions will be further explored for use as a myocardial imaging agent in humans.

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