LAT-1 Based Primary Breast Cancer Detection by [99m]Tc-Labeled DTPA-Bis-Methionine Scintimammography: First Results Using Indigenously Developed Single Vial Kit Preparation

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Abstract

Objective: To evaluate the diagnostic utility of a single vial ready to label with [99m]Tc kit preparation of DTPA-bis-methionine (DTPA-bis-MET) for the detection of primary breast cancer.

Methods: The conjugate (DTPA-bis-MET) was synthesized by covalently conjugating two molecules of methionine to DTPA and formulated as a single vial ready to label with [99m]Tc lyophilized kit preparations. Thirty female patients (mean age = 47.5 ± 11.8 years; range = 21–69 years) with radiological/clinical evidence of having primary breast carcinoma were subjected to [99m]Tc-methionine scintigraphy. The whole body (anterior and posterior) imaging was performed on all the patients at 5 minutes, 10 minutes, 1 hour, 2 hours, and 4 hours following an intravenous administration of 555–740 MBq radioactivity of [99m]Tc-methionine. In addition, scintimammography (static images; 256×256 matrix) at 1, 2, and 4 hours was also performed on all the patients.

Results: The resultant radiolabel, that is, [99m]Tc-DTPA-bis-MET, yielded high radiolabeling efficiency ($>97.0\%$), radiochemical purity (166–296 MBq/ μmol), and shelf life (>3 months). The radiotracer primarily gets excreted through the kidneys and localizes in the breast cancer lesions with high target-to-nontarget ratios. The mean $\pm$ SD ratios on the scan-positive lesions acquired at 1, 2, and 4 hours postinjection were 3.6 ± 0.48 , 3.10 ± 0.24 , and 2.5 ± 0.4 , respectively. [99m]Tc-methionine scintimammography demonstrated an excellent sensitivity and positive predictive value of 96.0% each for the detection of primary breast cancer.

Conclusion: Ready to label single vial kit formulations of DTPA-bis-MET can be easily synthesized as in-house production and conveniently used for the scintigraphic detection of breast cancer and other methionine-dependent tumors expressing the L-type amino acid transporter-1 receptor. The imaging technique thus could be a potential substitute for the conventional single-photon emission computed tomography (SPECT)-based tumor imaging agents, especially for tracers with nonspecific mitochondrial uptake. However, the diagnostic efficacy of [99m]Tc-methionine needs to be evaluated in a large cohort of patients through further multicentric trials.

Key words: [99m]Tc-methionine, breast scintimammography, cancer, single vial kit

Introduction

Breast cancer remains a major cause of mortality in women worldwide. Mammography despite being a key imaging method for breast cancer screening has its limitations of low diagnostic accuracy, particularly in the setting of fibrocystic and dense breast tissue in young patients.^{1–3} The various

tumor-imaging agents have been developed using single-photon emission computed tomography (SPECT)- or positron emission tomography (PET)-based technologies for an early detection of both primary and secondary malignancies.

Most commonly used SPECT tracers for breast imaging include [99m]Tc-diphosphonates, 201-thallium chloride ([201]Tl), and [99m]Tc-methoxyisobutylisonitrile ([99m]Tc-MIBI).

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However, a deeper understanding into the mechanism of uptake of these radiotracers has indicated either of these tracers, for example, [99m]Tc-MIBI, [201]Tl, [67]-Gallium represent hypermetabolism or cellular turnover, for example, 18-F-fluorodeoxyglucose ([18]F-FDG).⁴⁻⁶ The mechanism of uptake of these tracers actually makes them cell markers rather than tumor markers and their diagnostic utility is limited by low sensitivity and specificity for cancer detection.

Undoubtedly, FDG is a well-known glucose analog tracer for PET-based tumor imaging. [11C]-Methionine (MET), another PET tracer, is mainly incorporated into the newly synthesized proteins of tumor cells.⁷ [11C]-MET uptake can be used as an indirect, yet useful, indicator of the proliferative state of a tumor on baseline and follow-up PET scans.⁸⁻¹⁰ Various other non-FDG-PET tracers have been adopted as imaging probes to detect, stage, and monitor treatment efficacy in breast cancer. These probes are primarily targeted toward the identification of hormone receptors, growth factors, and angiogenesis factors such as ER, PR, HER2, and VEGFR, respectively.^{3,11} The role of [18]F-FDG-PET has been reported for primary cancer detection, locoregional and distant metastases, staging, as well as monitoring therapy response. However, heterogeneity of the disease may influence the results of FDG-PET imaging in breast cancer. FDG-positron emission mammography, which uses two opposite detector heads on either side of the breast to reduce the distance between the radiation source/s, provides a higher resolution than the conventional PET.¹² Likewise, contrast-enhanced FDG-PET and dual-point FDG-PET imaging is reported to have better sensitivity and specificity for the detection of small lesions in dense breasts.^{13,14} Despite the unparalleled imaging characteristics of FDG-PET, it still remains a nonspecific tracer as malignant/benign breast lesions, acute/chronic inflammatory lesions, and physiologic lactation may show a false-positive FDG uptake. In comparison with PET, SPECT imaging has the advantage of a wider range of conventional radiopharmaceuticals available in peripheral centers at a lower cost and gives a low radiation burden to the patients and the staff.

In the present study, the authors report the diagnostic utility using an indigenously developed single vial kit preparation of [99m]Tc-DTPA-bis-methionine (DTPA-bis-MET) for the scintigraphic detection of breast cancer. A detailed description of the chemical synthesis of the radiopharmaceutical, its preclinical evaluation and initial results using a non-kit formulation for the diagnosis in breast cancer patients, has been reported earlier.^{15,16}

Materials and Methods

Reconstitution of lyophilized methionine kits

The detailed methodology of the radiolabeling procedure, results of cell binding studies, and preclinical imaging studies performed using the resultant [99m]Tc-DTPA-bis-MET has been reported elsewhere.¹⁵ The radiolabeling scheme, as described in this study, was taken further to develop ready to label with [99m]Tc as single vial kit preparations of DTPA-bis-MET. A stock solution was prepared for the preparation for the kits and the volume of the solution was adjusted so that each vial contained a mixture of 10.0 mg of DTPA-bis-MET and 0.5 mg of stannous chloride. The vials were lyophilized

for 18 hours and sealed under vacuum. The ready to label single vial kits as lyophilized preparations were supplied by the Division of Cyclotron and Radiopharmaceutical Sciences, INMAS, DRDO, New Delhi, India. The kits were stored in a refrigerator at 4°C. Before use, the kits were brought to room temperature. About 740.0 MBq radioactivity of [99m]TcO₄⁻ (in 1.0 mL normal saline) was added to the kit constitutes by using a sterile syringe and needle and the mixture was allowed to stand for about 10 minutes. The radiolabeled preparation [99m]Tc-DTPA-bis-MET was filtered through a 0.22- μ m filter (Millipore, Bedford, MA). Before injecting the radiopharmaceutical into the patients, a sample from each reconstituted kit was subjected to various quality control tests, for example, radiolabeling efficiency, radiochemical purity, human serum stability, and sterility test. A detailed methodology describing these tests has been reported earlier.¹⁵

Scintigraphic studies

[99m]Tc-methionine scintimammography. Thirty female patients (mean age 47.5 ± 11.8 years, range 21–69 years) with clinical/radiological evidence of primary breast cancer disease were enrolled prospectively in the study. Twenty-four (24/30) patients had a history of invasive ductal carcinoma (IDC), 4 (4/30) of invasive lobular carcinoma, and the remaining 2 (2/30) patients of IDC *in situ* breast cancer disease. Pregnant or lactating patients or patients with known allergic or hypersensitivity to radiopharmaceuticals, if any, on previous occasions were excluded. The study protocol was cleared by the Institute's ethics committee and written and informed consent to participate in the study was obtained from each patient.

All the patients underwent [99m]Tc-methionine scintimammography using the dual-headed gamma camera (E Cam; Siemens, Erlangen, Germany) peaked at 140 keV (energy window $\pm 20.0\%$) and fitted with a low-energy/high-resolution collimator. Whole body (anterior and posterior) imaging was also performed in all the patients at 5 minutes, 10 minutes, 1 hour, 2 hours, and 4 hours following an intravenous administration of 555–740 MBq radioactivity of [99m]Tc-methionine. In addition, scintimammography (static images; 256 $\times$ 256 matrix) at 1, 2, and 4 hours was also performed in all the patients using a scintimammography pellet. The scintimammography data were subjected to a semiquantitative analysis to calculate the lesion-to-background ratio (LBR) of the radiotracer as a function of imaging time intervals. For the purpose of calculating the LBR, a region of interest (ROI) was drawn around the breast area showing focal uptake of [99m]Tc-methionine and an equally sized ROI was placed on the contralateral normal breast. In 2 patients with bilateral breast carcinoma, a perilesional background ROI was used and LBR was calculated in all the positive lesions.

Results

The kit formulation method involved the synthesis of conjugation (DTPA-bis-MET) by covalently conjugating two molecules of methionine to DTPA. The lyophilized conjugate (DTPA-bis-MET) preparation was then formulated into single vial ready to label (with [99m]Tc) kit preparations and used in the present study. The resultant radiolabel obtained after reconstituting the kit was subjected

to various *in vitro* tests like radiochemical purity, human serum stability, cytotoxicity studies, cell uptake/efflux studies, blood kinetics, dose escalation and biodistribution studies. These results were comparable with the corresponding results obtained with the non-kit formulations reported previously.¹⁵

The mean labeling efficiency of [^{99m}Tc]-DTPA-bis-MET kit preparations used in the present study was estimated to be $97.0\% \pm 1.5\%$ ($n=35$). The radiochemical purity was in the range of 166–296 MBq/ μ mol. The radiopharmaceutical was stable up to 24 hours. The sterility test was negative as there was no evidence of any bacterial growth/colony formation on the agar plates kept for 14 days. No untoward reaction was observed in any of the patients following intravenous administration of the radiotracer.

[^{99m}Tc]-methionine biodistribution studies

The whole body anterior and posterior scans acquired at 5 minutes, 10 minutes, 1 hour, and 2 hours were visually interpreted for the organs normal biodistribution of [^{99m}Tc]-methionine. A representative anterior whole body image is presented in Figure 1. A significant amount of the injected radiopharmaceutical is seen concentrating in the kidneys at 5 and 10 minutes and draining into the urinary bladder by 10 minutes. An almost complete wash out of the radiopharmaceutical was seen from the kidneys to the bladder by 2 hours, indicating that the tracer gets excreted primarily by the renal route. The heart and buccal mucosal activity seen at 5 and 10 minutes had completely cleared off by 1 hour. A minimal intestinal/thyroidal radioactivity was seen in the serial whole body images.

[^{99m}Tc]-methionine scintimammography

A total of 32 breast lesions (14 left-sided and 18 right-sided breast lesions; at least 1 lesion/patient; 2 patients had

bilateral lesions) in the 30 patients enrolled in the study were analyzed. Histopathology was positive for the evidence of breast cancer in 29/32 and negative in the remaining 3/32 lesions. [^{99m}Tc]-methionine scintimammography findings were positive in 29/32 lesions. A correlation with HP analysis demonstrated true-positive and true-negative scan findings for 28 and 2 lesions, respectively. On the other hand, false-positive and false-negative scan findings were observed in 2 patients (one lesion/patient), respectively. The sensitivity and positive predictive values of [^{99m}Tc]-methionine scintimammography were estimated to be 96.0%. On the other hand, the specificity and negative predictive values of the technique were 67.0%.

A semiquantitative analysis of the positive (29 lesions) breast lesions (with focal concentration of [^{99m}Tc]-methionine) revealed a mean LBR of 3.6 ± 0.48 , 3.10 ± 0.24 , and 2.5 ± 0.4 derived on the scintimammography images acquired at 1, 2, and 4 hours, respectively. On the other hand, a significantly lower LBR of 1.7 ± 0.07 ($p=0.001$), 1.6 ± 0.40 ($p=0.01$), and 1.4 ± 0.25 ($p=0.05$) at 1, 2, and 4 hours, respectively, was observed for the negative lesions (only three lesions). The LBR analysis on the methionine scintimammography imaging data is presented in Table 1.

A representative scintimammographic scan of a primary breast cancer lesion (true positive) showing a consistently rising LBR of the radiotracer in the left breast until 4 hours after imaging is presented in Figure 2. A true-negative lesion (with histopathologically negative/benign pathology) having no focal uptake in either breast is presented in Figure 3.

Discussion

Malignant transformation increases the use of amino acids for energy, protein synthesis, and cell division as the tumor cells have been found to have overexpressed transporter systems.¹⁷ Most of the amino acids are taken up by the tumor cells through an energy-independent L-type amino acid

FIG. 1. Whole body (anterior) images acquired at 0 minute, 10 minutes, 1 hour, and 2 hours following intravenous injection of 555.0 MBq of [^{99m}Tc]-DTPA-bis-methionine showing normal biodistribution of the tracer. The arrows indicate the focal uptake of the radiotracer in the left breast lesion.

TABLE 1. A SEMIQUANTITATIVE ANALYSIS OF THE POSITIVE AND NEGATIVE BREAST LESIONS DERIVED ON THE SCINTIMAMMOGRAPHY IMAGES ACQUIRED AT 1-, 2-, AND 4-HOUR TIME INTERVALS, RESPECTIVELY, AFTER ADMINISTRATION OF THE RADIOTRACER

Imaging time intervals	Mean lesion-to-background ratio $\pm$ SD		p-Value
	Positive lesions (n = 29)	Negative lesions (n = 3)	
1 hour	3.58 $\pm$ 0.48	1.7 $\pm$ 0.07	0.001
2 hours	3.10 $\pm$ 0.24	1.6 $\pm$ 0.40	0.01
4 hours	2.54 $\pm$ 0.36	1.44 $\pm$ 0.25	0.05

transporter (LAT-1) system and a sodium-dependent transporter system A and B.¹⁸ The amino acids are retained in the tumor cells due to their higher metabolic activities, including incorporation into proteins, than most of the normal cells.¹⁷ A variety of [11]C- and [18]F-labeled amino acids have been used for PET applications in oncology.¹⁹⁻²¹ [11C]MET has

FIG. 2. [99m]Tc-DTPA-bis-methionine-scintimammography images (acquired at 1, 2, and 4 hours) in a 66-year-old patient demonstrating increased tracer uptake in the left breast (positive lesion) as compared with the right breast (negative benign lesion, shown by arrows).

FIG. 3. [99m]Tc-DTPA-bis-methionine-scintimammography images (acquired at 1, 2, and 4 hours) in a 25-year-old patient demonstrating bilateral normal breasts having no abnormal focal uptake of the radiotracer.

been widely used in the detection of brain, head and neck, lung, and breast cancer, as well as lymphoma.²¹ It can cross the blood-brain barrier and is incorporated mainly into proteins and also into lipid, RNA, and DNA. [11C]-MET-PET imaging is more sensitive to radiotherapy compared to FDG and is useful for monitoring treatment of cancer.²¹ However, the short half-life of [11]C remains a limitation, and therefore, [99m]Tc-labeled amino acid (with longer half-life) may provide a better substitute for the accurate characterization of the tumor lesions.

In the present study, the authors evaluated the diagnostic utility of a single vial kit preparation of [99m]Tc-labeled methionine kits for the detection of primary breast carcinoma. An excellent labeling efficiency (mean value of 97.0% $\pm$ 1.5%) was observed for the reconstituted [99m]Tc-labeled kit

preparations ($n=30$) used in the present study. It has been reported earlier by this research group that the freshly prepared non-kit (nonlyophilized) form of the preparation of conjugated methionine had a radiolabeling efficiency of 96.3%, radiochemical purity of >99.0%, and specific activity of 4500–8000 $\mu\text{Ci}/\mu\text{mol}$ (166.0–296 MBq/ μmol), and a radiochromatogram showed a single peak eluting at 6.4 minutes by a C-18 reverse-phase column.¹⁵ Furthermore, the preclinical evaluation has shown that the radiopharmaceutical preferentially accumulates in the tumors induced in animal models using various cell lines, for example, U-87 MG, BMG, and EAT tumor cell lines.¹⁵ The biodistribution studies indicated that [99m]Tc-methionine gets excreted from the body primarily by renal excretion. A minimal activity of the radiotracer was seen in the intestine and thyroid (Fig. 1). As no heart activity was seen after 1 hour, scintimammography using [99m]Tc-methionine acquired at 1 hour and beyond, may thus offer an excellent technique (high target-to-background ratio) for breast tumor imaging in the light of negligible mediastinum (background) activity.

The authors performed [99m]Tc-methionine scintimammography in 30 breast cancer patients as a part of the clinical trials with the radiopharmaceutical in the form of lyophilized and ready to label single vial preparations. It was observed that the radiopharmaceutical showed a focal uptake in the primary site of histopathologically proven breast carcinoma. A semiquantitative analysis has shown that the LBR of the radiotracer on the scan-positive lesions was significantly higher than that on the negative lesions. The imaging technique exhibited excellent sensitivity and PPV (96% each); however, the specificity and NPV were low (67.0% each). [99m]Tc-methionine scan findings in the present study were in consonance with the findings reported earlier.¹⁶ The findings of low specificity and NPV (67.0%) may be due to the recruitment of mainly (29/32=90%) histopathologically proven breast cancer patients in the present study. The main aim of the study was to see the diagnostic utility of the ready to label [99m]Tc-methionine kits as a routine scintigraphic procedure in peripheral nuclear medicine centers, which neither have the expertise for synthesis of newer radiotracers nor have the expensive PET/cyclotron facility.

This study's findings indicated that the synthesis technique could be formulated easily into single vial ready to label (with [99m]Tc) kits and is an excellent technique for breast cancer imaging as a routine nuclear medicine diagnostic procedure. In addition, a semiquantitative analysis of the lesions may add further confidence in scan interpretation, as was evident in the present study, where the LBR of the true-positive lesions was significantly higher than the LBR of true-negative lesions. However, a small number of negative lesions (only three lesions) remains the limitation of this study. Therefore, for deriving the cutoff values to characterize the lesions as positive or negative, studies in a larger cohort of patients through multicentric trials are needed. Therefore, the diagnostic efficacy of [99m]Tc-methionine scintimammography needs to be checked through prospective studies in histopathologically negative or in asymptomatic patients with incidentally detected breast lesions. Interestingly, it was also noted that the mean LBR of true-positive lesions at 1 hour was significant ($p=0.001$) than the negative lesions. Hence, a confident interpretation

of the tumoral tracer uptake can be made as early as 1 hour and the study duration can be shortened, which in turn may lead to patient convenience and increase in the number of patients who can undergo the investigations in a busy clinic.

As compared to other conventional diagnostic modalities, a wide variety of nuclear medicine tracers available for breast cancer imaging offers an obvious advantage both for molecular diagnosis and determination of the therapeutic options. To date, only FDG-PET has been recommended by the European and American guidelines for the evaluation of distant metastases in locally advanced breast cancer and to monitor treatment response in primary inoperable and distant metastatic breast cancer disease.¹¹ However, it is reported that with the use of only [18F]-FDG/[18F]-fluoride PET, the widely accepted standards of the disease activity are often missed out. In addition, among the entire spectrum of the available SPECT imaging agents, although [99m]Tc-sestamibi is approved for breast imaging, it is not part of the NCCN guidelines (version 3.2012) due to a lack of consensus/expert opinion on its diagnostic efficacy.¹¹ This warrants the need for development of SPECT-based radiopharmaceuticals with better diagnostic efficacy than the existing ones. It has been reported that various cancer types, including breast cancer, express LAT-1 and the expression was closely correlated with CD98 expression, angiogenesis, and cell proliferation.²² Therefore, it may be of further interest to use this radiotracer in the metastatic workup of cancer patients targeting the LAT-1 expression on various cancer types.

It is highlighted that ready to label single vial kit formulations of DTPA-bis-MET can be easily synthesized in-house and can be conveniently used for the scintigraphic detection of breast cancer and other methionine-dependent tumors. As the mechanism of uptake of the radiotracer is through the overexpressed LAT-1 receptors on the malignant cells, the imaging technique thus could be a potential substitute for the conventional SPECT-based tumor imaging agents, especially for tracers with nonspecific mitochondrial uptake. However, the diagnostic efficacy of [99m]Tc-methionine needs to be evaluated in a large cohort of breast cancer patients (with locoregional/distant metastases) through further multicentric trials being undertaken prospectively after obtaining the encouraging results for imaging of primary breast cancer using [99m]Tc-methionine as single vial kit preparations. This approach could be of special significance in the developing countries in view of the low cost of the conventional nuclear medicine procedures as compared to the expensive PET/cyclotron technology. The imaging technique also could be of particular interest in the detection of brain tumors.

Disclosure Statement

There are no existing financial conflicts.

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