International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 – 7587
Print ISSN 2249 - 7595
Research Article

A NEW RADIOPHARMACETICAL FOR THE DETECTION OF SENTINEL NODE: 99mTc-5-FU

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ABSTRACT
Objective: The purpose of the study is to evaluate 99mTc-5 FU for the detection of sentinel node by Nuclear Imaging (gamma camera and gamma probe). Material and methods: We have done a study of 3 patients with carcinoma breast who referred for the sentinel node imaging and gamma probe detection. Nuclear Imaging was done by SPECT system and Gamma probe detection done by gamma probe for the detection of sentinel node. Results: Out of three in two patients we have observed the radiocolloid movement and detection of sentinel node has been detected. Conclusion: We have observed that 99mTc-5-FU radiocolloid is a potential radiopharmaceutical for the detection of sentinel node after having further characterization and other tests.

Keywords: Radiocolloid, 99mTc-Fluorouracil (99mTc-5-FU), Scintigraphy, Gamma probe.

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INTRODUCTION

The sentinel node imaging and biopsy has been used in clinical practice for breast cancer, head and neck cancer, melanoma, penile cancer and gynecological cancers. Sentinel lymph node is the first regional lymph node that drains the lymph from the primary tumor. It is potentially the first node to receive the seedlings of lymph-borne metastatic cells [1]. Lymphoscintigraphy (LS) allows the surgeon to easily identify and biopsy the sentinel lymph node. This method identifies the sentinel node but cannot determine if it is involved with cancer. At present, this technique guides the surgeon to the sentinel node, and sentinel node biopsy has been reported as a safe and accurate method of screening axillary nodes [2, 3].

Access this article online

Home page: http://ijmca.com/

DOI: http://dx.doi.org/10.21276/ijmca.2018.8.1.2

Quick Response code

Received:12.02.18 **Revised**:22.02.18 **Accepted**:28.02.18

Radiotracer kinetics plays a big role in the selection suitable radiopharmaceutical and planning the operative procedure. The comparison of the time taken by the radio colloid, complex and colloid to show peak uptake and height of detection efficiency is important from detection point of view. The radiopharmaceuticals 99mTc-labeled sulfur colloid (99mTc-SC) is used for sentinel node imaging in nuclear medicine facility. Using a microfiltration process, smaller particles of filtered 99mTc-SC (99mTc-SC) can be used to assess for detection the sentinel node of tumor drainage for patients with breast cancer. (5)

OBJECTIVE

The objective of the study is to do evaluation of 99mTc-5-FU for the detection of sentinel node.

MATERIAL AND METHOD

Chemicals

5-Flurouracil was a kind of gift from Aura laboratories Pvt.Ltd. L-cysteine hydrochloride monohydrate purchased from Finar Chemical Ltd.

Stannous chloride purchased from Finar Chemical Ltd. NaOH a kind of gift from Aura laboratories Pvt. Ltd. Column generator 99mMo/99mTc generator, dry generator supplied by Pars Tech, Iran. All other chemicals were also analytical and reagent grade used from radiopharmacy lab of the department. E.g. distilled water and normal saline etc

Instruments

Syringe filters imported from Merck Millipore Ltd, Ireland. ITLC and PC kit from BRIT, Mumbai. Symbia SPECT System, Siemens, Germany. Gamma Probe of crystal photonics has been used. Other instruments and equipments used from Radio pharmacy lab of nuclear medicine department of Bhagwan Mahaveer Cancer Hospital and Research Centre, Jaipur.

RADIOLABELING PROCEDURES

Direct labeling approach: This approach involved use of radioisotope, drug of interest and an effective reducing agent. For direct labelling of 5-Flurouracil with 99mTc, stannous chloride was used as reducing agent. Fresh pertechnetate ([99m] TcO4 -) eluted from ([99] Molybdenum- [99m] Technetium Column Generator (Pars Tech, Iran) was used for the labelling procedures. The variable concentrations 1 mg to 20 mg of stannous chloride (Sncl(2).2H(2)O), different pH (2–9) conditions, and variable incubation times (2 minutes, 5 minutes, 10 minutes, 20 minutes, and 30 minutes) were tested. [99m] Tc-5-Flurouracil was prepared by dissolving 100mg of 5-Flurouracil in 100 mL distilled water followed by addition of a standardized concentration of 2mg stannous chloride dihydrate and pH was marked to 7.2.

The contents were filtered through a 0.22 um membrane filter (Millipore) into a sterile vial. Aprox 40.0 MBq radioactivity of pertechnetate was added to the 1 ml of mixture and incubated for 15-20 minutes. The resultant radioligand [99m] Tc-5-Flurouracil was then subjected to various quality control tests.

Radiopharmaceutical kit Preparation steps

5mg 5-fluorouracil was dissolved in 100 ml of distilled water with continuous stirring. 50 mg of L-cysteine hydrochloride monohydrate was added in it. Afterwards, 2mg $SnCl_2.2H_2O$ was added and pH was maintained at 7.2 by using 5N NaOH, 2N NaOH, 1N NaOH and 0.1N NaOH. The product was passed through a 0.22 µm membrane filter. 1 ml/kit of resultant solution were dispensed in sterilized serum vials. 10 mCi in 0.5 ml of $Na^{99m}TcO4$ eluted from $^{99}Mo/^{99}Tc$ generator added to the kit (1 ml) and incubated at room temperature for 20 min.

QUALITY CONTROL

In Vitro Quality Control Procedures

Radiochemical purity (RCP) of the 99m Tc-labeled 5-Fluorouracil studied by using two simple chromatographic techniques, e.g., Instant Thin Layer Chromatography (ITLC) and Paper Chromatography (3mm whatman paper). For quality control two methods were used viz. Paper chromatography and Instant thin layer chromatography.

Paper Chromatography: Placed approximately 1 ml of acetone into one 10-mI glass vial and 1 ml of 0.9% NaCI into an identical vial. Loaded a spot of radiopharmaceutical at the bottom of the Whatman paper chromatography strip and marked the position of the same with a pencil line.

Developed paper strip in acetone solution for free pertechnate and in 0.9% NaCI solvent for free hydrolyzed Tc, until solvent front migrated to top. Cut strips into sections. Counted all sections for activity (per unit time) using a gamma counter.

Instant Thin Layer Chromatography: Radiochemical purity of the labeled complex was determined by instant thin layer chromatography (ITLC) using 100% acetone and 0.9% sodium chloride as solvents. Briefly, 20.0 micro liter of radiometal complex was dropped onto the ITLC strip at the marked origin point and put into the solvent chamber at room temperature. The percent labeling of [99m]Tc-5-FU was calculated at 15 minutes, 1 hour, 4 hours, and 24 hours by ITLC method. The percentage of free pertechnetate, hydrolyzed pertechnetate, and bound pertechnetate was calculated.

The QC method can determine the percentage fraction of radioisotope bound to ligand, hydrolyzed portion, and amount of free pertechnetate. Acetone used as a mobile phase for paper chromatography and saline was used for ITLC. Small aliquots from the reconstituted kit were spotted on the respective strips. The strips, after elution, were cut in fractions of 1 cm and counted for radioactivity in a gamma imaging system/counter.

RADIOMETAL STRUCTURE: Figure.2

Patients: Total 3 patients, female, biopsy proven, Stage I and II, breast carcinoma. Scintigraphic sentinel node localization considered in women who have a biopsy-proven carcinoma of the breast in whom definitive surgery and axillary node clearance is planned and on whom there are no palpable axillary lymph nodes [4].

We have taken ethic committee approval for the study and clinical use of 99mTc-5-FU and a written consent has been taken from all three patients.

Patient Preparation

All the patients are counseled about the sentinel node imaging and other procedure. We have taken a written consent from all the patients who have under gone the sentinel node imaging and gamma probe procedure. NBM in the management of breast carcinoma and alternative methods of staging disease and should understand that by agreeing to SN Imaging and SLNB, they may also need to undergo axillary dissection.

There is no special preparation for the procedure, other than preoperative restrictions if sentinel node imaging is being performed on the same day of surgery. Patient should wear a front-opening patient gown. As per routine practice, the patient's clothes above waste and all relevant metallic items (coins, jewellery etc.) removed before injections.

Radiopharmaceutical Preparation and Adminis tered Activity

We have injected 11.1 - 18.5 MBq (300-500 mircocuri) doses. Administration of 99mTc-5-FU Colloid were injections planned according the dose category. Small volume of injection 0.1 to 0.2 ml injected at each with the help of 25 G needle. Peritumoral injections were preferred.

We cover injection site with gauze/cotton wool ball while withdrawing the needle to avoid skin contamination and seal with a small waterproof plaster immediately the needle is removed. Patient advised to massage injection site for 3-4 minutes to promote radiotracer kinetics and movement of tracer into lymphatic channels and basin nodes.

Image Acquisition

Image acquisition by gamma camera, detector kept in L Mode, LEHR collimator and Pixel matrix size of 128 X 128 or 256 x 256. Patient should be supine, with arm on affected side completely abducted to allow head of gamma camera to come as close as possible to the axilla. Use an injection stand or other equivalent support for the arm. This position should mirror that of the patient at surgery especially for breast cases.

Early dynamic images within 30 minutes with 15 seconds per frame and followed by delayed static images to analysis the tracer kinetics.

Skin marking

Skin marking is done with the help of gamma camera image and pencil or point source marker.

Figure 1. Radiolableing of 99mTc-5-FU

Figure 2. Putative structure of 99mTc-5-Flurouracil

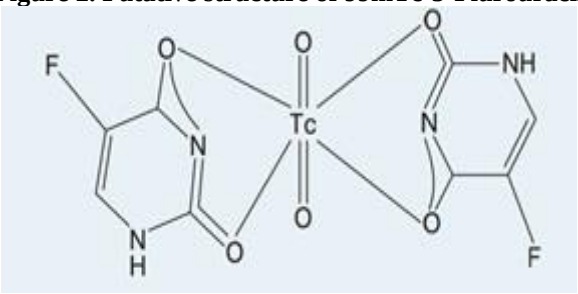

Image 3. Scintigraphy and Skin Marking

Image 4. Gamma Probe: (a) Sentinel nodes localization, (b) Counts survey in dissected nodes

RESULTS AND DISCUSSION

Radio Chemical Purity (RCP)

The radiochemical purity test has been done by Paper Chromatorygrahy (PC) and ITLC (BRIT, Mumbai). The radiochemical purity was found above 97.50%. pH of the radio colloid was checked by digital pH meter and found 5.5-7.5.

In the presented study we have done on three female patients of ca breast for the detection of sentinel node imaging by 99mTc-5-FU. The below points can be discussed on the basic of the obtained patient images and data.

It is observed that the radio colloid of 99mTc-5-FU shown the uptake in sentinel node and got detected by gamma camera and gamma probe. We have got positive results in two patients and nodes were got detected. In one patient result was negative there was no movement of radiocolloid has been detected.

The tracer kinetics plays a very useful role in the selection of colloid and planning of sentinel node operative procedures. It also can be helpful in the time management and time calibration for the subjected patient.

Even though the radiation levels are low for sentinel node procedures, good radiation handling practices should still be adopted in all cases during surgery, specimen transportation and pathological processing to ensure that the ALARA (As Low as Reasonably Achievable). (16)

CONCLUSION

In the presented study we have found that radiocolloid of the 99mTc-5-FU can be used for the detection of sentinel node imaging. The 99mTc-5-FUcolloid was shown the movement and positive results in two patients it remained unmoved in one patient with negative results.

The radiocolloid nature, tracer kinetics for filtered and unfiltered and detection efficiency to be studied in future work for the wide speared use of the 99mTc-5-FU radiocolloid.

REFERENCES

1. Keshtgar MRS, Ell PJ. Sentinel lymph node detection and imaging. *Eur J Nucl Med*, 26, 1999, 57–67.
2. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, et al., 2003. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med*, 349, 2003, 546–53.
3. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S, Galimberti V, et al. Sentinel-lymph-node biopsy as a staging procedure in breast cancer: update of a randomized controlled study. *Lancet Oncol*, 7, 2006, 983–90
4. De Cicco C, Cremonesi M, Luini A, Bartolomei M, Grana C, Prisco G, et al. Lymphoscintigraphy and radioguided biopsy of the sentinel axillary node in breast cancer. *J Nucl Med*, 39, 1998, 2080–4.
5. Maggie M. Michenfelder1, Lucas J. Bartlett1, Douglas W. Mahoney, Thomas J. Herold1, and Joseph C. Hung. Particle-Size and Radiochemical Purity Evaluations of Filtered 99mTc-Sulfur Colloid Prepared with Different Heating Times. *J Nucl Med Technol*, 42, 2014, 283–288.
6. www.pharmacopeia.cn/v29240/usp29nf24s0_m80790, 2013. Technetium Tc 99m sulfur colloid injection. U.S. Pharmacopeia website. Available only to paid subscribers at: html. Accessed January 23, 2013.
7. Larson SM, NeIp WB. Radiopharmacology of a simplified technetium-99m colloid preparation for photoscanning. *J Nuci Med*, 7, 1996, 8l7-826.
8. Mariani G, Moresco L, Viale G, et al. Radioguided sentinel lymph node biopsy in breast cancer surgery. *J Nucl Med*, 42, 2001, 1198–1215.
9. Veronesi U, Paganelli G, Viale G, et al. A randomized comparison of sentinelnode biopsy with routine axillary dissection in breast cancer. *N Engl J Med*, 349, 2003, 546–553.
10. Uren RF, Howman-Giles RB, Shaw HM, Thompson JF, Mccarthy WH, 1993. Lymphoscintigraphy in high-risk melanoma of the trunk: predicting draining node groups, defining lymphatic channels and locating the sentinel node. *J Nucl Med*, 34, 1993, 1435–1440.
11. Mariani G, Gipponi M, Moresco L, et al. Radioguided sentinel lymph node biopsy in malignant cutaneous melanoma. *J Nucl Med*, 43, 2013, 811–827
12. Belhocine TZ, Prefontaine M, Lanvin D, et al. Added-value of SPECT/CT to lymphatic mapping and sentinel lymphadenectomy in gynaecological cancers. *Am J Nucl Med Mol Imaging*, 3, 2013, 182–193.
13. Vermeeren L, Valdés Olmos RA, Meinhardt W, et al. Value of SPECT/CT for detection and anatomic localization of sentinel lymph nodes before laparoscopic sentinel node lymphadenectomy in prostate carcinoma. *J Nucl Med*, 50, 2009, 865–870.
14. Vermeeren L, Meinhardt W, Bex A, et al. Paraaortic sentinel lymph nodes: toward optimal detection and intraoperative localization using SPECT/CT and intraoperative real-time imaging. *J Nucl Med*, 51, 2010, 376–382
15. Giuliano AE, Kirgan DM, Guenther JM, Morton DL, 1994. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. *Ann Surg*, 220, 1994, 391– 398.

16. Coventry BJ, Collins PJ, Kollias J, Bochner M, Rodgers N, Gill PG, Chatterton BE and Farshid G. Ensuring Radiation Safety to Staff in Lymphatic Tracing and Sentinel Lymph Node Biopsy Surgery. *J Nucl Med Radiat Ther*, 2012, S:2

1.

Cite this article:
Kan Singh Shekhawat1, Lokhande RS, Bhagat JK. A New Radiopharmacetical for the Detection of Sentinel Node: 99mTc-5-FU. *International Journal of Medicinal Chemistry & Analysis*, 2018;8(1):6-10.
DOI: http://dx.doi.org/10.21276/ijmca.2018.8.1.2