

A NEW MONOCLONAL ANTIBODY RADIOPHARMACEUTICAL FOR RADIOIMMUNOSCINTIGRAPHY OF BREAST CANCER: DIRECT LABELING OF ANTIBODY AND ITS QUALITY CONTROL

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ABSTRACT

Radioimmunosintigraphy (RIS) has found widespread clinical application in tumor diagnosis. The antibody (Ab) PR81 is a new murine anti-MUC1 monoclonal antibody (MAb) against human breast carcinoma. In this study a very simple, rapid and efficient method for labeling of this MAb with ^{99m}Tc, particularly suitable for development of a 'kit' is described. The reduction of Ab was performed with 2-mercaptoethanol (2-ME) at a molar ratio of 2000:1 (2-ME:MAb) and the reduced Ab was labeled with ^{99m}Tc *via* methylene diphosphonate (MDP) as a transchelator. The labeling efficiency which was determined by instant thin layer chromatography (ITLC) was 94.2%±2.3. Radiocolloids measured by cellulose nitrate electrophoresis were 2.5%±1.7. *In vitro* stability of the labeled product in human serum which was measured by gel filtration chromatography (FPLC) was 70%±5.7 over 24 hr. The integrity of labeled MAb was checked by means of SDS-PAGE and no significant fragmentation was observed. The results of the cell-binding studies showed that both labeled and unlabeled PR81 were able to compete for binding to MCF 7 cells. Biodistribution studies were performed in normal BALB/c mice at 4 and 24 hrs post-injection and no important accumulation was observed in vital organs. These results show that the new radiopharmaceutical may be considered as a promising candidate for imaging of breast cancer.

Keywords: Breast cancer; MUC1; Monoclonal antibody; Technetium-99m-radiolabeling

INTRODUCTION

Radioimmunosintigraphy is a recent approach in diagnosis of cancer. It takes advantages of the antibody specificity of tumor surface antigens for targeting active tumor cells and of the emitted radiation from suitable radioisotope, as a means of imaging (1, 2). Human epithelial mucin, MUC1, is a highly glycosylated transmembrane protein that is normally present on the luminal surface of secretory glands. This glycoprotein is aberrantly over expressed in adenocarcinomas including 80% of the breast cancers and represents a useful target for RIS (3). Therefore, use of antibodies directed against this antigen might increase the early diagnosis of the breast cancers in such patients. There have been reports about some imaging methods in animal models and human with breast

cancers using many murine anti-MUC1 MAbs (4-8). Although the results have been promising and persuading, none of them have yet entered in the clinical uses. Recently a new murine anti-MUC1 MAb against human breast carcinoma has been reported (9). The new monoclonal antibody was assigned as PR81. Due to high specific reactivity and its characteristics, this MAb is a promising agent in diagnosis and therapy of the breast cancer.

There are two general methods for labeling of an antibody with ^{99m}Tc. In the direct methods the reduced antibody reacts with ^{99m}Tc (10-14) and in the indirect methods a bifunctional chelating agent is used to conjugate the antibody to ^{99m}Tc (15-17). Direct methods are efficient and adaptable for kit type radiolabeling procedure. Nevertheless, the

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use of a reducing agent may affect immunoreactivity of the reduced antibody and the labeled antibody could be unstable for evaluation. The direct method for radiolabeling of reduced MABs with ^{99m}Tc in the presence of MDP, (18,19) has proved as a good procedure to obtain high-labeling efficiency and a stable labeled MAB. In the ^{99m}Tc -MDP methods the disulfide bridges of the molecule are reduced by addition of an excess of 2-mercaptoethanol (2-ME) as reducing agent. After purification to eliminate the excess of 2-ME, the reduced antibody is labeled with ^{99m}Tc via Sn^{2+} reduction of pertechnetate, using MDP as a weak competing ligand. These conditions are conveniently provided by the use of a conventional radiopharmaceutical bone-scanning kit. A general scheme is shown in figure 1.

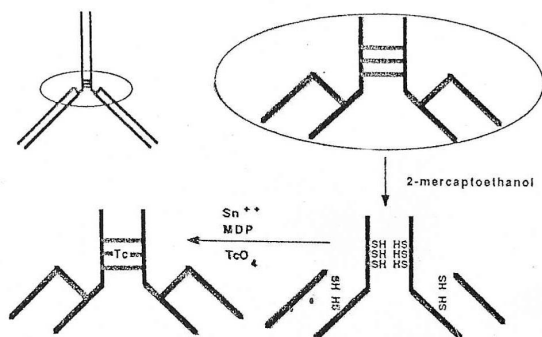


Figure 1. General scheme for direct radiolabeling procedure. Reduction of MAB by 2-ME and binding of ^{99m}Tc to it via MDP

In the previous study the use of direct method for radiolabeling of PR81 with ^{99m}Tc in the presence of stannous tartrate as a transchelator (20) was described. The labeling efficiency, immunoreactivity and *in vitro* stability (measured at room temperature) of the produced complex was good. Nevertheless the method was not simple and rapid and not very suitable for development of a simple, rapid kit. In this study by the use of methylene diphosphonate as a transchelator and efficient labeling method, was developed which is suitable to prepare a kit. Furthermore in order to check the characteristics of the new preparation before its application in RIS studies of the breast cancer, the stability of the labeled antibody in human serum and its binding ability to MCF 7 cells were studied.

MATERIAL AND METHODS

Monoclonal antibody

The antibody PR81 was supplied by the department of biotechnology, school of medical sciences, Tarbiat modarres university, Tehran, Iran

in vials containing 1 ml of sterile PBS with an antibody concentration of 5 mg/ml.

Cell line

A MUC1 positive cell line, MCF 7, was purchased from Pasteur Institute (Tehran, Iran) and was grown in RPMI 1640 having 10% FCS, 100 units/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 2 mM glutamine, and 5 $\mu\text{g}/\text{liter}$ insulin.

Radiolabeling

The reduction of MAB was performed by 2-ME at the molar ratio of 2000:1 (2-ME-MAB) according to the results obtained from the previous study (20). The reduced antibody was then purified on a 1×10 cm G-50 Sephadex (Sigma) column, pre-equilibrated with PBS.

The ability of the reduced antibody to label with ^{99m}Tc was assessed as follows:

1) An MDP bone-scanning kit (Nuclear Research Center, Atomic Energy Organization of Iran, Tehran, Iran) containing 6.25 mg medronate, 0.8 mg stannous chloride and 4.5 mg NaCl and 0.5 mg ascorbic acid was reconstituted with 5 ml of 0.9% saline.

2) For each mg of the reduced MAB 5, 10, 20, 50, 80 and 100 μl of the MDP solution was added to 100-300 $\mu\text{g}/\text{ml}$ of antibody followed by addition of required amount of ^{99m}Tc in the form of sodium pertechnetate (20 μCi ^{99m}Tc per 1 μg Ab) which was eluted from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (Nuclear Research Center, Atomic Energy Organization of Iran, Tehran, Iran) by 0.9% NaCl.

Quality control

The quality control tests were performed as described in the previous study (20). Briefly, the labeling efficiency was determined by ITLC. Radiocolloids were measured by cellulose nitrate electrophoresis. The integrity of the labeled MAB was checked by means of SDS-PAGE. Biodistribution studies were performed in normal BALB/c mice at 4 and 24 hrs post-injection.

In vitro stability in human serum

Size-exclusion FPLC analysis on a Superose 12 HR 10/30 in which 0.01 M Na_2HPO_4 , pH 7.0 eluent was used as eluent was employed to estimate the stability of ^{99m}Tc -PR81 in fresh human serum at 37°C after 24 hrs (21). The labeled antibody was added at a concentration of about 10 $\mu\text{g}/\text{ml}$ and 100 μl of each sample were analyzed at 1.0 ml/min flow rate monitored by UV detector. Fractions of 1.0 ml were collected and counted separately in a γ -counter.

Cell-binding assay

MCF 7 cells (3×10^4 cells/well) were plated on a sterile 96-well plate and grown to confluency (22). The cells were fixed by ice-cold methanol at -20°C

for 10 min. Each well (100 μ l) contained 15 KBq of ^{99m}Tc -PR81 and 0-1000 $\mu\text{g/ml}$ of cold PR81 MAb. After incubation for 3 hrs at room temperature, the wells were washed three times with 180 μ l of cold 0.01 M PBS (pH 7.4). Then, 100 μ l of 1 N NaOH was added to each well to solubilize the cells at 60 $^{\circ}\text{C}$ for 30 min. The solubilized cells (80 μ l) from each well were counted for ^{99m}Tc radioactivity using a γ -counter.

RESULTS

Radiolabeling

Paper chromatography showed that 20 μ l of MDP solution at concentration of 250mg/ml per each mg of reduced MAb was appropriate to obtain the labeling efficiency of $94\% \pm 2.3$.

Results of cellulose nitrate electrophoresis showed that radiocolloids have $2.5\% \pm 1.7$ of total radioactivity (Fig. 2).

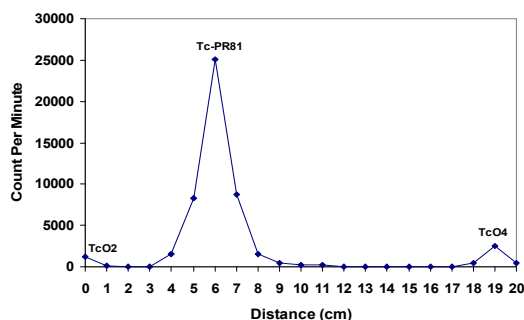


Figure 2. Radioelectrophoregram of ^{99m}Tc -PR81 complex on cellulose nitrate for measuring radiocolloids (TcO_2)

In vitro stability in human serum

Figure 3 presents the size-exclusion FPLC radiochromatogram of the labeled samples of human serum at 37 $^{\circ}\text{C}$ after 24 hrs of incubation (in buffer [A] and human serum [B]). The results showed that more than 70% of total radioactivity was related to a homogeneous peak with a retention volume similar to that of the native antibody. The results demonstrated the stability of the radiolabeled antibody, and its suitability for *in vivo* diagnosis.

Cell-binding assay

The affinity of the ^{99m}Tc -PR81 for the target human MUC1 was determined by the cell-binding assay. The binding of the labeled antibody to the surface of human breast adenocarcinoma cells, MCF 7 cell line, was increased by decrease in concentration of cold PR81 (Figure4). The ability of this complex to compete with cold antibody to bind to the cell receptors shows a high tagging of ^{99m}Tc -PR81 onto the human breast cancer cells.

Integrity

The results of SDS-PAGE are presented in figure 5. As it can be observed, the labeling procedure did not lead to very significant modification of the MAb structure.

Biodistribution

Figure 6 shows the result of ^{99m}Tc -PR81 biodistribution in mice at 4 and 24 hrs after IV administration expressed as percentage of injected dose per gram of tissue (%ID/g). The results show that there was no significant accumulation of radioactive antibody in vital organs.

DISCUSSION

It was previously found that IgG antibodies of reasonably high affinity ($\times 10^{-8}$) are optimal for both imaging and therapy (23). In this way, PR81, which exhibited high affinity ($2.19 \times 10^{-8} \text{ M}^{-1}$) towards some MUC1 positive cell line (MCF-7, BT-20 and T-47D) may be suitable for *in vivo* tumor targeting and imaging (9). Therefore its labeling with a radioisotope followed by its quality control are the first steps towards the use of this antibody in RIS studies.

In theoretical terms, the ideal method for the labeling of antibodies is probably the one based on the conjugation of a pre-labeled technetium chelate to the antibody molecule (17). Such an approach gives maximum control over the labeling process and radiochemical purity and should be applicable to a wide variety of protein molecules. However, such methods, in their present stage of development have a number of disadvantages, notably the lengthy preparation times and the required high level of chemical expertise. Furthermore in any antibody labeling method, the aim is to produce a label with high stability using a procedure with minimal effect the immunoreactivity of the antibody. When radionuclides with short half-lives, such as ^{99m}Tc , are used it is also desirable that the labeling procedure to be rapid, in order to minimize losses due to radioactive decay, and to be simple in the way that the technique may be widely applicable (19, 21, 24).

The method described above fulfills all these requirements. The procedure is simple, rapid, and antibody is easily prepared in a lyophilized formulation which results in labeling efficiency higher than 94%, which negates the need for post-labeling purification. The method is particularly suitable for development of a 'kit,' which further makes the process further simple and permits the use of these radiopharmaceuticals in centers where limited number of pharmaceutical expertise are present.

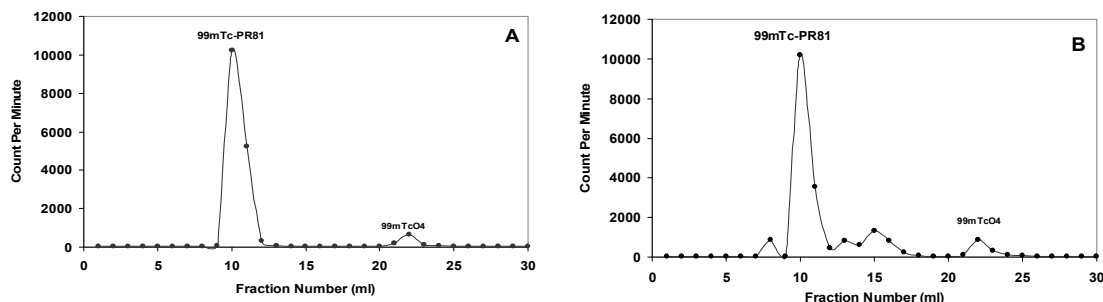


Figure 3. Size exclusion FPLC radiochromatograms of ^{99m}Tc-PR81 in buffer (A) and after 24 hrs of incubation in 37°C human serum (B).

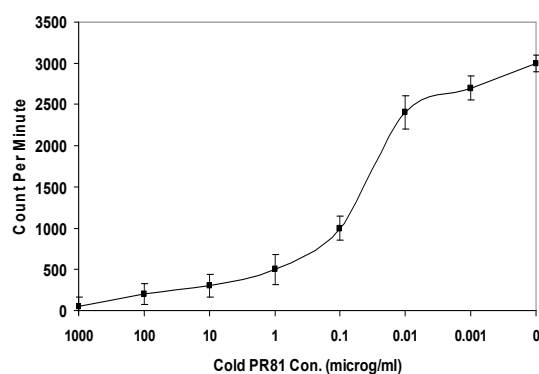


Figure 4. Cell-binding assay with methanol-fixed MCF 7 cells as the solid-phase source of human MUC1 and ^{99m}Tc-PR81 as the tracer ligand. The competitive displacement of binding of the ^{99m}Tc-PR81 to the MCF 7 cells was measured in the presence of 0-1000 µg/ml concentration of cold PR81.

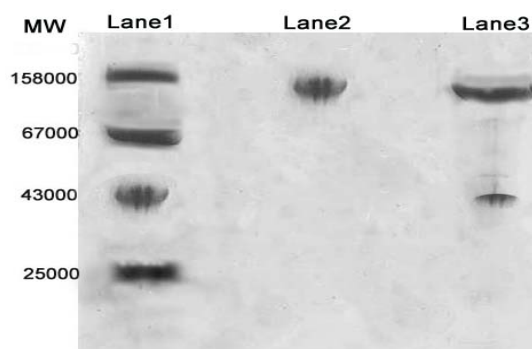


Figure 5. SDS-PAGE with Coomassie blue staining of molecular weight standards (lane 1), native PR81 (lane 2) and ^{99m}Tc-PR81 (lane 3).

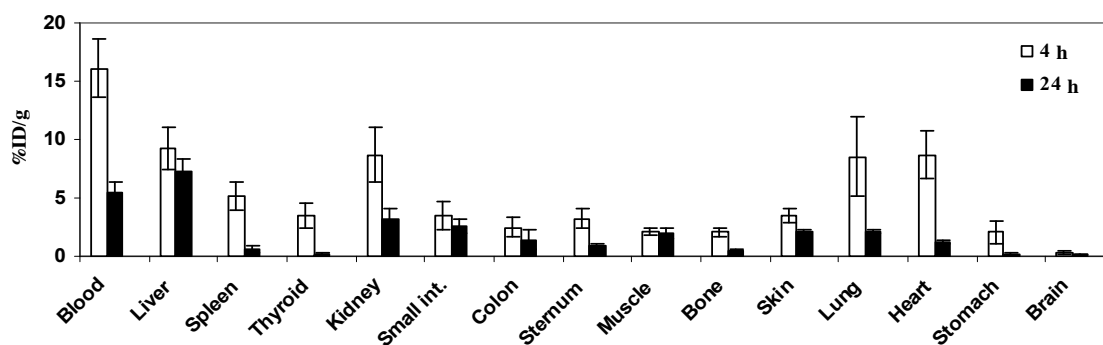


Figure 6. Biodistribution data of ^{99m}Tc-PR81 in normal BALB/c mice performed at 4 and 24 hrs post-injection and expressed as percentage of injected dose per gram of tissue (%ID/g).

This research was also conducted to investigate the characteristics of the new product which ultimately will be used for RIS studies of breast cancer. The ability of MAbs to bind to the surface of cancer cells and their stabilities in human serum are generally considered to be useful parameters in selection of 'strong Abs' for use in RIS studies. According to the results obtained in our study ^{99m}Tc-PR81 showed high stability in human serum

and high tagging onto the MCF 7 cells which is a human breast carcinoma cell line. Furthermore the lack of significant accumulation of the preparation in vital organs of the animal model and its existence in blood 24 hrs post-injection, are very important factors for a radiopharmaceutical for nuclear medicine applications, and as a result ^{99m}Tc-PR81 may be considered as a promising candidate for imaging of breast cancer.

CONCLUSION

These results show that, by the use of the Schwarz method for radiolabeling of MAb PR81, is achieved a labeling yield higher than 94%. High stability of the complex in human serum, its very good binding to the surface of human breast

adenocarcinoma cells and good behavior in *in vivo* studies demonstrated the high quality of the preparation for RIS studies of breast cancers. This methodology could also be used for kit formulation.

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