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Radiochemical synthesis and preliminary evaluation of anti-bladder cancer monoclonal antibody BDI-1 labeled with rhenium-188

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Summary

The anti-human bladder cancer monoclonal antibody BDI-1 was radiolabeled with rhenium-188 by direct labeling methods using $SnCl_2$ as reductant and MDP as stannous stabilizer or by indirect method using NHS-ECM ester as bifunctional chelator. Radiochemical yields of $30 \pm 7.23\%$ and $87.4 \pm 5.67\%$ and radiochemical purity of more than 95% were achieved. The inmmunoreactive fraction was 58.7%. The results showed that radionuclide ^{188}Re might be employed using the same labeling methodology with ^{99m}Tc labeling of BDI-1 for radiommunoimaging (RII) and radioimmunotherapy (RIT) and that may be useful in radioimmunodetection and RIT of human bladder carcinoma in vivo. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: Rhenium-188; Bladder carcinoma; Monoclonal antibody BDI-1; Radioimmunoimaging (RII); Radioimmunotherapy (RIT)

1. Introduction

Bladder and ureteral cancers are common urological malignancies and represent the most common urological cancers in the world. The traditional diagnosis is established by cystoscopy and biopsies of bladder mucosa and additional examinations such as ultrasonography,

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radiography and computed axial tomography (CT) may also contribute to the diagnosis. However, these methods are all non-specific and have variable sensitivity; for flat or *in situ* carcinoma, small solid lesions, and bladder cancer metastatic foci, quantitative diagnosis is relatively difficult. On the other hand, the characteristics of bladder cancer constitute the superficial features for most primary tumors and the high recurrent rate (50–70%) after surgery. Currently, there are no ideal therapeutic means besides chemotherapy and the use of bacillus Calmette–Guerin (BCG) vaccine administered intravesically due to whole body toxic reaction and poor efficacy.[1,2] For these reasons, we are evaluating approaches with RII using ^{188}Re–BDI-1 in a template in a new radioimmunotherapeutic approach.

With the development of hybridoma technology the use of radiolabeled monoclonal antibodies (MoAbs) for radioimmunodetection and RIT has undergone extensive investigation. For example, there are several approaches to the diagnosis of bladder cancer using radiolabeled monoclonal antibodies which have demonstrated that RII may be valuable for the diagnosis of bladder cancer.[3–5] The BDI-1 is an anti-bladder carcinoma cell line BIU-87 monoclonal antibody which has been prepared in our hospital. In our previous work, ^{131}I-BDI-1 and ^{99m}Tc-BDI-1 have been shown to have good affinity to human bladder carcinoma cell line BIU-87 and have shown excellent localization in nude mice bearing human bladder cancer xenograft.[6–8] Recently, the therapeutic radioisotopes of rhenium, rhenium-186 and rhenium-188, have been proposed for use in RII, especially in RIT due to their excellent therapeutic physical characteristics and chemical similarities to technetium. This is due to the hypothesis that, if an acceptable ^{99m}Tc labeling of MoAbs is available, ^{188}Re might be used with the same labeling methodology for RII and RIT.

In this paper, we describe the preparation of anti-bladder tumor monoclonal antibody (BDI-1) labeled with radionuclide rhenium-188 (^{188}Re–BDI-1) for use in radioimmuno-guided treatment of bladder cancer and have evaluated its property of locating bladder cancer.

Experimental

Preparation of monoclonal antibody BDI-1

The BDI-1 monoclonal antibody was obtained from a hybridoma produced by fusing spleen cells from mice immunized against the

bladder carcinoma cell line BIU-87 with SP 2/0 mouse myeloma. It was purified from mouse ascitic fluid by protein A Sepharose CL-4B affinity chromatography. The monoclonal antibody was characterized by indirect immunofluoresence assay and ABC–ELISA immunohistochemical staining which demonstrated that BDI-1 has a strong binding reaction with bladder transitional cell carcinoma tissue and BIU-87 and E-J bladder carcinoma cell lines but there was no reaction with normal bladder tissue and other normal human tissues.[9]

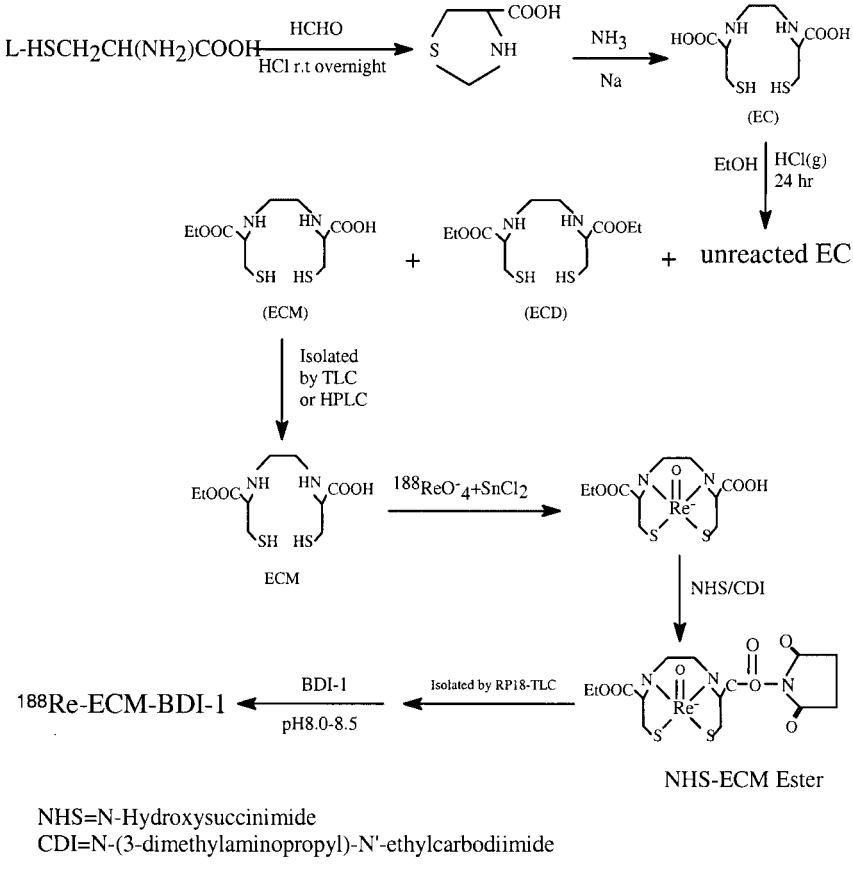
Immunohistochemistry was used to study the expression of the antigen in tumors with different grades and stages in 45 specimens of TCC (transitional cellular carcinoma) by virtue of monoclonal antibody BDI-1 bindings. The bindings of BDI-1 was strong, when the tumor grading was high, but no significant differences were observed between the different stages ($p > 0.05$). The expression of antigen, which was related to MoAb BDI-1, was correlated with the grade of tumor. The higher the tumor grading, the higher was the expression of antigen.

Radiochemical synthesis of ^{188}Re–BDI-1

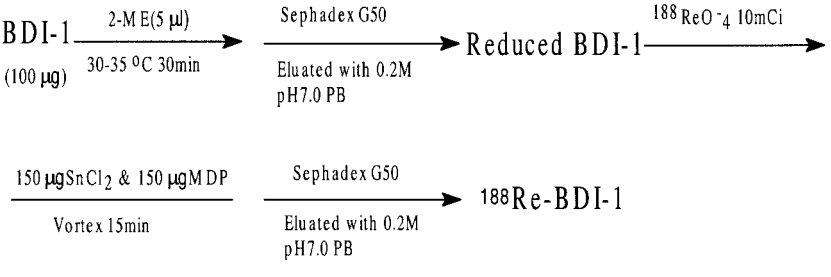
[^{188}Re]-perrhenate was obtained from ^{188}W/^{188}Re generator which was purchased from Syncor Company. ^{188}Re isotope has a 155 keV γ-emission suitable for RII, and has a 2.21 MeV β-emission suitable for RIT. MDP kit was purchased from Shanghai Hongqi Paramaceutical Factory and 2-mercaptoethanol (2-ME) was purchased from Merck Company. IR was recorded on Perkin Elmer.

BDI-1 reduced by 2-mercaptoethanol was labeled with Re-188 either by indirect method with ^{188}Re-NHS-ECM ester (**Scheme 1**) or by direct labeling with $^{188}ReO_4^-$ using $SnCl_2$ as reductant and MDP as stannous stabilizer (**Scheme 2**).

Formaldehyde (HCHO) was added in L-cysteine dissolved in aqueous HCl reacted overnight at r.t., and then purified to produce L-thiazolidine-4-carboxylic acid. EC was obtained by reducing the L-thiazolidine-carboxylic acid with sodium in liquid ammonia and then reacted with ethanol under reflux for 24 h to obtain a mixture which was then isolated by HPLC to obtain ECM, IR ($CHCl_3$): 2977 cm^{-1} (CH_3), 1230 cm^{-1} (CO), 1076 cm^{-1} (C–N), 940 cm^{-1} (O–H). ECM was added to a solution of ^{188}Re sodium perrhenate 37–740 MBq, followed by the



Scheme 1. Synthetic route of indirect labeling BDI-1 with rhenium-188



Scheme 2. Synthetic route of direct labeling BDI-1 with rhenium-188

addition of 0.2 ml of freshly prepared solution of stannous chloride (0.1 mg) in 0.1 N HCl of pH = 4.0. ^{188}Re-ECM was determined by paper chromatography using two developing systems. System 1 used $CHCl_3 : CH_3OH = 9 : 1$ (v/v) as eluent, R_f (ECM, ECD) = 0.9–1.0, R_f

($^{99m}TcO_4^-$, EC) = 0–0.1. System 2 used acetone as eluent, R_f (ECM, ECD) = 0–0.1, R_f (ECD, $^{99m}TcO_4^-$) = 0.9–1.0. ^{188}Re-ECM reacted with *N*-hydroxysuccinimide using *N*-(3-dimethylaminopropyl)-*N'*-ethyl carbodiimide as condensing agent to produce ^{188}Re–NHS–ECM ester. After purification with RP18-TCL, the pre-ester was coupled to BDI-1 under pH 8.0–8.5 to obtain ^{188}Re–ECM–BDI-1. The radiolabeling yields of the antibody were determined on silica gel using an eluent of 0.9% NaCl. R_f (MoAbs) = 0–0.1, R_f ($^{188}ReO_4^-$) = 0.9–1.0.

The direct labeling approach was based on the method previously described by Griffiths *et al.*[10] with some modifications. Briefly, ^{188}Re–BDI-1 was prepared by 2-mercaptoethanol (2-ME) direct reducing method. Five µl 2-ME was added to 100 µl BDI-1, then reacted for 30 min at 30–34°C to reduce the disulfide bonds of the antibody to mercapto groups. After the reduced antibody was purified by Sephadex G-50, 148–296 MBq (4–8 mCi) $^{188}ReO_4^-$, 40 µl dissolved MDP kit by 2 ml saline and 135 µg $SnCl_2$ in HCl aqueous were added and shaken for 15 min at room temperature. The labeled antibody was purified by Sephadex G-50 and passed through a 0.22 microfilter to obtain sterile product. The radiochemical purity was controlled by paper chromatography

Immunoreactivity studies

Immunoreactivity studies were performed using the method described in the literature[11]. Briefly, six serial 1 : 2 dilutions of two aliquots of human bladder cancer cell line BIU-87 starting at 5×10^6 cell/ml were made. Then, the cell suspensions were centrifuged and resolved in 100 µl 1% BSA. To 1 of 2 aliquots of cells, unlabled antibody was added to a final concentration of 50 µg/ml and the cells were incubated for 2 h to saturate the binding sites for the subsequent measurement of nonspecific binding of radiolabled antibody. Radiolabeled antibody at a concentration of 40 µg/ml in 100 µl 1% BSA was added to each cell suspension. After 2 h of incubation, the radioactivity (total applied) was measured. The cells were centrifuged, washed three times with 1% BSA, then the radioactivity (specific binding) was measured. The data were plotted by total applied radioactivity over specific binding as a function of the inverse of cell concentration. The immunoreactive fraction was determined by means of linear extrapolation to infinite cell concentration.

Radioimmunoimaging

Radioimmunoimaging of three nude mice bearing human bladder cancer xenografts was performed at 24 h after i.v. injection of 11.1 MBq of ^{188}Re–BDI-1 using a General Electric STARCAM 300AC γ camera equipped with a low-energy high-resolution collimator. Analog and digital images were acquired from the dorsa view with the collimator 10 cm from the animal. Images were acquired with an energy peak of 150 keV and matrix of 128×128 from approximately 50000 accumulation counts resulting in imaging times ranging from 5 to 15 min. Digital images were normalized with a General Electric STARCAM computer to produce visually similar levels of activity in the central torso. Tumor and whole body regions of interest were identified on each mouse image.

Results and discussion

A new anti-bladder cancer monoclonal antibody BDI-1 prepared by ourselves was reduced by 2-mercaptoethanol. Rhenium-188 labeling with reduced antibody was carried out either by indirect method with ^{188}Re–NHS-ECM or by direct labeling with $^{188}ReO_4^-$ using $SnCl_2$ as reductant and MDP as stannous stabilizer. Labeling yields at different $SnCl_2$ concentration, reaction time and pH values were studied.

Both the direct labeling method[10] and indirect labeling method[12] can be used for labeling antibodies with radioactive rhenium. In our study, radiolabeling yields of $30 \pm 7.23\%$ by the direct method and $87.4 \pm 5.76\%$ by the indirect method were demonstrated and more than 95% radiochemical purity of both the means was achieved. Direct labeling is a simple method and widely used, but it is not very effective in labeling BDI-1 with Re-188. The indirect method with NHS–ECM was developed in our study and higher radiolabeling yield was achieved as ECM could be labeled under high acidity. The immunoreactive fraction of ^{188}Re–BDI-1 was more than 58.7%. The optimum radiolabeling conditions considering both yields and immunoreactivity require further investigation

RII of nude mice bearing human bladder cancer xenografts showed significant radioactive accumulation on the tumor at 24 h postinjection (**Figure 1**). The result demonstrated the excellent immunoreactivity of ^{188}Re–BDI-1 *in vivo*.

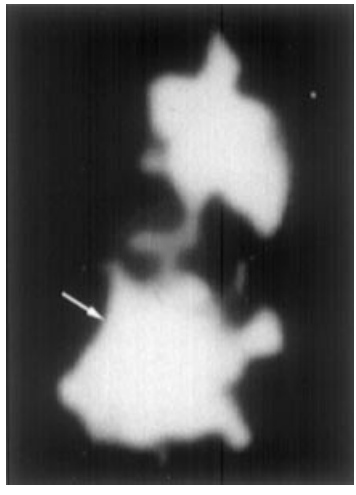


Figure 1. RII of nude mice bearing human bladder cancer xenografts. Radioimmunoimaging in nude mice bearing human bladder human bladder cancer xenografts was performed at 24 h after intravenous injection of 11.1 MBq of ^{188}Re–BDI-1 using a General Electric STARCAM 300AC γ camera equipped with a low-energy high resolution collimated. It demonstrated the selected radioactive accumulation on tumor (in arrowhead). The radioactivity in the liver can also be seen

Bladder cancer is one of the most common urological tumors and postoperative recurrence is common, which necessitates the examination of methods in the early diagnosis of bladder cancer for treatment planning and prognosis. RII may be used to diagnose bladder tumor, which is associated with the following two factors. The first factor includes the expression of tumor antigen, the immunoactivity and specificity of monoclonal antibody (MoAbs). The second factor includes the availability and cost effectiveness of the selected radioisotopes. The physical properties of Rhenium-188 (155 keV γ-emission suitable for RII, and 2.12 MeV β-emission suitable for RIT) make it an excellent candidate as a radionuclide for radioimmunotherapy when it is covalently chelated to MoAbs. In comparison with ^{131}I, ^{188}Re has many favorable nuclear properties such as suitable γ-energy for scintigraphic imaging and strong β-energy for intraradiation therapy of tumors and is more and more widely used in therapeutic nuclear medicine.[13] Furthermore, ^{188}Re can be easily obtained from the generator of ^{188}W/^{188}Re,[14] which is more available in routine clinical practice.

Conclusions

We developed a new potential radiopharmaceutial rhenium-188–BDI-1, for radioimmunotherapy, permitting its successful labeling with radioactive rhenium. The labeling compound showed higher radiolabeling yields of 87 $\pm$ 5.76% from indirect labeling method with ^{188}Re–NHS–ECM than that of 30 $\pm$ 7.23% from direct labeling method using 2-mercaptoethanol reduction, and has an available immunoreactivity of 57.8%. RII in animals bearing human bladder cancer xenograft has demonstrated significant radioactive accumulation in the tumor. The preliminary results shown by our study also justify the development of radioimmunoconjugates for RIT, especially to provide a novel means for radionuclide intraradiation therapy of bladder cancer by intravesical perfusion of ^{188}Re–BDI-1.

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