Annals of Nuclear Medicine Vol. 12, No. 2, 83–88, 1998

Localization of colorectal carcinoma by rhenium-188-labeled B72.3 antibody in xenografted mice

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In order to evaluate the feasibility of ^{188}Re-labeled antibodies for radioimmunotargeting, monoclonal antibody B72.3, recognizing TAG-72, expressed on the surface membranes of colorectal cancer cells, was directly labeled with ^{188}Re, obtained from a ^{188}W/^{188}Re generator, using stannous tartrate and compared with ^{125}I-labeled B72.3. As a control, a human IgG was also radiolabeled with ^{188}Re and ^{125}I. Prepared antibodies for ^{188}Re labeling could be stored as kits. Biodistribution was determined in nude mice inoculated with human colorectal carcinoma LoVo. Labeling efficiency and immunoreactivity of ^{188}Re-B72.3 were 80.3% and 64.7%, respectively. ^{188}Re-B72.3 localized specifically in the LoVo tumors. Although the absolute tumor accumulation level of ^{188}Re-B72.3 was lower than ^{125}I-B72.3, ^{188}Re-B72.3 demonstrated higher tumor-to-blood contrast than the ^{125}I-labeled counterpart, 2.04 ± 0.44 vs. 1.05 ± 0.28 at 96 hours, because of fast clearance from the blood. ^{188}Re-B72.3 seemed efficient for the imaging and therapy of colorectal carcinoma.

Key words: rhenium-188, xenograft, B72.3, colorectal carcinoma

INTRODUCTION

THE RADIOISOTOPES of rhenium, ^{186}Re and ^{188}Re, have currently been tested for radiolabeling of antibodies.[1-4] ^{186}Re has a 1.1 MeV β-emission complemented with a 137 keV γ-emission and a half-life of 90.64 hours. ^{188}Re has a maximum β-emission of 2.11 MeV and 155 keV of gamma photons, and the half-life is 16.98 hours. Both provide imageable gamma photons for scintigraphy, and appropriate β particles for radioimmunotherapy.

Beaumier et al. reported the effectiveness of ^{186}Re-labeled antibodies for the treatment of small-cell lung carcinoma in a mouse model.[5] Further, ^{186}Re-labeled HEDP and EDTMP have been used as bone seeking agents for pain relief therapy in breast or prostate cancer patients with bone metastasis,[6,7] but as ^{186}Re contains a variable amount of carrier rhenium which can be an obstacle in labeling antibodies, ^{186}Re is less desirable for the antibody-guided internal radiotherapy. On the other hand, ^{188}Re is readily available as carrier-free sodium perrhenate in isotonic saline from a ^{188}W/^{188}Re generator system.

In this study, a murine monoclonal antibody (Mab) B72.3 was labeled with ^{188}Re by a direct method based upon reduction of the antibody molecule and binding of ^{188}Re to the thiol group. B72.3, recognizing TAG (tumor associated glycoprotein)-72 antigen expressed on the cell surface of colorectal carcinomas,[8,9] has been widely used in the diagnosis of colorectal cancer patients.[10,11] In this study, the efficacy of ^{188}Re-B72.3 was estimated by conducting biodistribution studies in nude mice inoculated with human colon carcinoma LoVo cells.

Received October 16, 1997, revision accepted December 11, 1997.

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MATERIALS AND METHODS

Monoclonal antibody

B72.3, a murine IgG_1, was obtained from Sterling Winthrop (Malvern, PA, USA). This antibody was raised by immunizing mice with membrane enriched fractions of a human breast carcinoma metastatic to the liver.[8] It reacts with the human tumor antigen TAG-72, a high molecular weight glycoprotein with characteristics of a mucin, and expressed in colon cancers, breast cancers and ovarian cancers. On the basis of immunopathological examinations, TAG-72 is expressed in up to 85% of colon cancers, 70% of breast cancers, and 95% of ovarian cancers, but it shows minimal or no expression in normal adult tissue.[12,13]

Antibody preparation

B72.3 was prepared for ^{188}Re labeling as previously reported.[14] Briefly, the antibody was dialyzed overnight against either saline or buffer (maltose, 5%; potassium hydrogen phthalate, 40 mM; potassium sodium tartrate, 10 mM; Glycine, 0.3 M; pH = 5.6). The antibody was then reduced in this same buffer made to be 2 mM with stannous tartrate. The reduction reaction was carried out under the atmosphere for 21 hours at room temperature. The reduced or prepared antibody was then purified on PD-10 columns (Pharmacia LKB Biotechnology, Uppsala, Sweden). The protein concentration of the column eluate was determined by Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA), and the antibody solution was then diluted to 2 mg/m*l* with buffer. This was mixed 1 : 1 with buffer containing 8 mM stannous tartrate so that the final concentration was 1 mg reduced antibody per m*l* in 4 mM stannous tartrate. One-m*l* aliquots of this solution were lyophilized, sealed under argon and stored at 4°C until needed. As a control antibody, human IgG (Bayer, Leverkusen, Germany) was prepared by the same methods as B72.3.

^{188}Re labeling

The ^{188}W/^{188}Re generator was supplied by Oak Ridge National Laboratory (Oak Ridge, TN, USA).[15] The generator was eluted with saline, and the radioactivity of the perrhenate obtained was assayed with a dose calibrator. One m*l* of perrhenate solution was added to a vial containing the lyophilized prepared antibody. The vial was gently swirled to dissolve the solids and initiate the labeling reaction. The reaction was allowed to proceed overnight (17 hours) at room temperature.

^{125}I labeling

B72.3 and human IgG were radioiodinated by the Chloramine-T method. Briefly, 40 μg of purified Mabs in 0.3 M phosphate buffer (PB), pH 7.5 and 11.1 MBq of ^{125}I were mixed with 2.5 μg of chloramine-T dissolved in 0.3 M PB. After 5 minutes of reaction, radiolabeled Mabs were separated from free radioiodine by PD-10 gel chromatography.

Immunoreactivity

Immunoreactivity was determined by an affinity thin layer chromatography. The solution containing the radiolabeled antibody was diluted 1 : 20 with 50% newborn calf serum and 10 μ*l* aliquots spotted onto a pair of thin layer chromatography strips (RhoChekIITM, RhoMed Inc. Albuquerque, NM, USA).[16-18] One strip was positive and had a band of antigen on the strip immediately upstream from the origin. The second strip was a negative control, which did not have an antigen band. The strips were developed in PBS containing 4% ethanol. Radioactivity bound to the immunoreactive antibody remained within the band of antigen in the positive strip. Some preparations contained radiochemical impurities, such as radiocolloids, which remained at the origin on both the positive and negative strips. Thus to determine net immunoreactivity, the percentage of the radioactivity at the origin of the positive strip was corrected by subtracting the percentage of radioactivity at the origin of the negative strip. Immunoreactivity of ^{188}Re-labeled and ^{125}I-labeled antibodies was estimated by this method.

Cell binding

The human colorectal cell line LoVo[19] was cultured in RPMI 1640 culture medium (GIBCO BRL, Gaithersburg, MD, USA) supplemented with 1 mM glutamine and 10% fetal calf serum. To determine the binding to LoVo cells, ^{125}I- and ^{188}Re-B72.3 diluted with 0.5% bovine serum albumin (Immuno GmbH, Heidelberg, Germany) in saline were incubated with increasing concentrations of LoVo cells in 5.7 × 46 mm microcentrifuge tubes for 1 hour at 4°C. After centrifugation at 1,500 × *g*, the tubes were washed with saline and cut. The radioactivity bound to cells was counted in a well-type gamma counter. Specific binding to cells was calculated by subtracting the nonspecific binding in the tubes in which 20 μg of unlabeled B72.3 was added.

Xenografts and mice

For the study in mice, 5 to 7 week-old female NMRI nu/nu athymic mice were purchased from Harlan (Borchen, Germany). 1×10^7 of LoVo cells per mouse were implanted by s.c. inoculation into the flanks of mice. Xenografts were allowed to grow 3 to 4 weeks after inoculation. The weight of xenografts used in this study (n = 50) was 0.35 ± 0.19 g and there was no significant difference among groups.

Biodistribution study

Groups of 5 mice bearing LoVo xenografts per time point were given 200 kBq of ^{188}Re-B72.3 and 37 kBq of ^{125}I-B72.3 at the same time via the tail vein. At 30 min, 6 hours 1, 2, 3 and 4 days after injection, the mice were sacrificed.

The radioactivity of tumors and selected organs was determined with a well-type gamma counter. Biodistribution of ^{188}Re-labeled and ^{125}I-labeled human IgG was also determined at 1, 2, 3 and 4 days after injection.

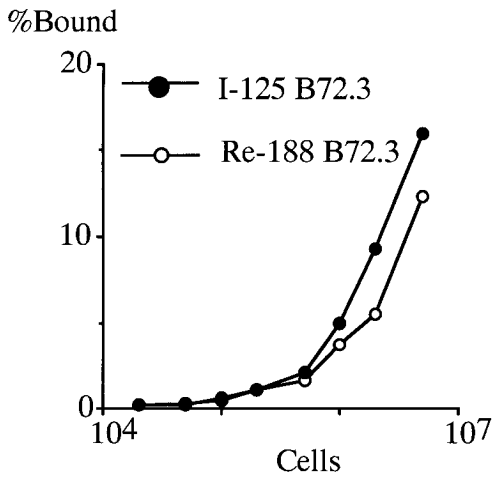

Fig. 1 Binding assay of ^{188}Re- and ^{125}I-labeled B72.3 to LoVo cells. Non-specific binding was subtracted. ^{188}Re-labeled B72.3 demonstrated a binding similar to ^{125}I-labeled B72.3.

RESULTS

Labeling efficiency and immunoreactivity, which was determined by thin layer chromatography, of ^{188}Re-labeled B72.3 were 80.3% and 64.7%. On the other hand, labeling efficiency and immunoreactivity of ^{125}I-labeled 72.3 were 53.8% and 72.4%. Final specific activities of 5.62 mCi/mg and 5.38 mCi/mg were achieved for ^{188}Re-labeled B72.3 and ^{125}I-labeled B72.3, respectively.

The cell binding assay demonstrated specific binding of ^{188}Re- and ^{125}I-labeled B72.3 to LoVo cells (Figure 1), and there was no significant difference between the two counterparts.

Biodistribution of ^{188}Re-labeled B72.3 and human IgG showed that ^{188}Re-labeled localized in the tumor well (Figure 2). ^{188}Re-B72.3 in the tumor reached the maximum accumulation of 3.49 ± 0.89%ID/g at 48 hours after injection. ^{125}I-B72.3 localized also in the LoVo tumor with the peak accumulation of 7.16 ± 1.96%ID/g at 48 hours (Figure 3).

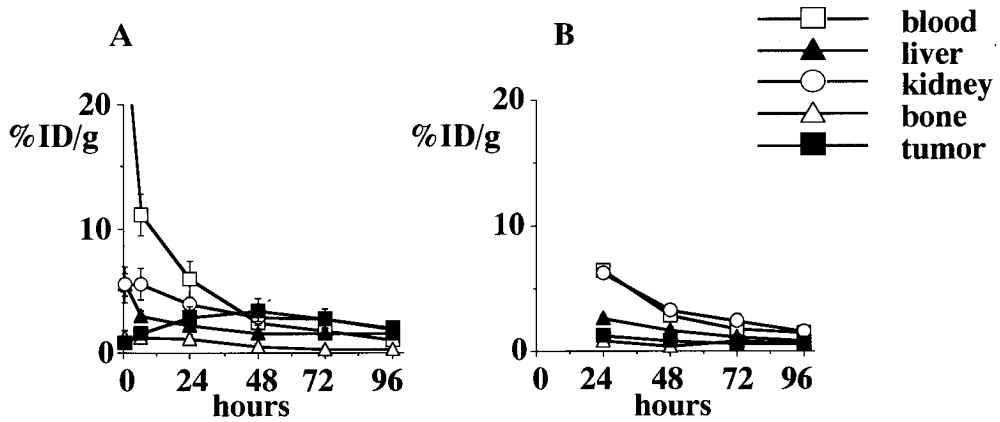

Fig. 2 Biodistribution of ^{188}Re-B72.3 (A) and ^{188}Re-human IgG (B) in athymic mice bearing LoVo cells. ^{188}Re-labeled antibodies cleared quickly from the blood and normal organs.

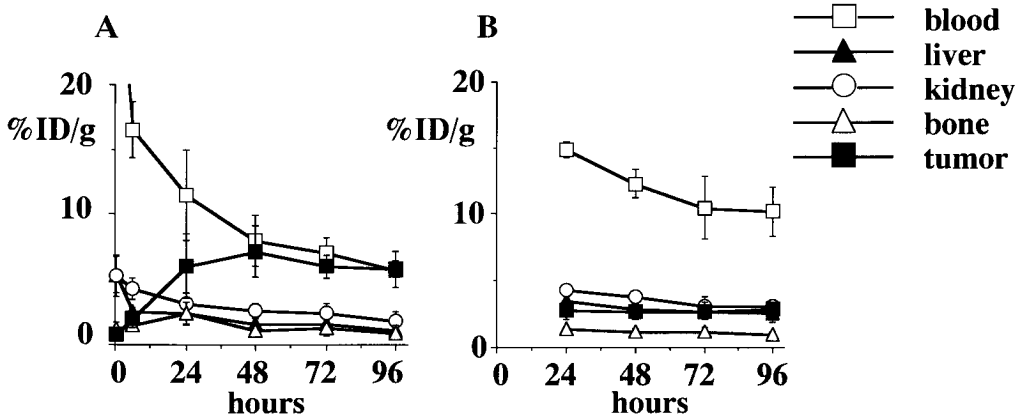

Fig. 3 Biodistribution of ^{125}I-B72.3 (A) and ^{125}I-human IgG (B) in athymic mice bearing LoVo.

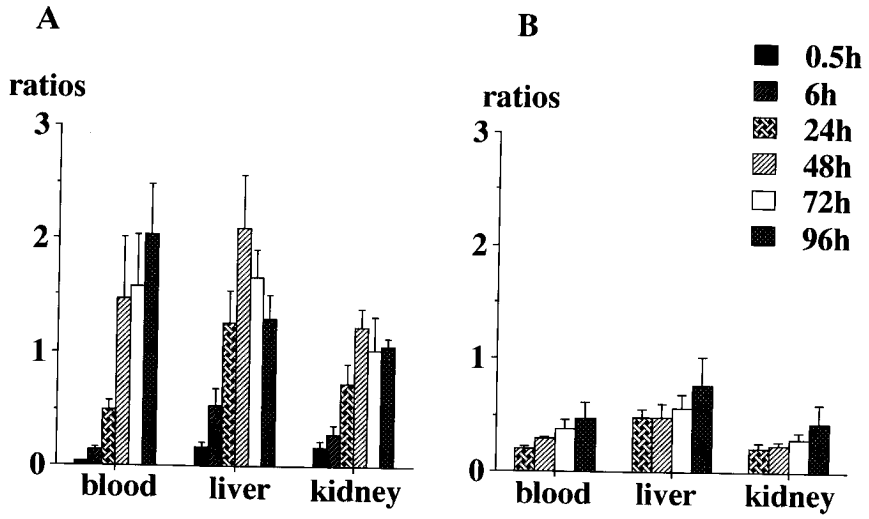

Fig. 4 Tumor-to-organ ratios of ^{188}Re-B72.3 (A) and ^{188}Re-human IgG (B) in athymic mice bearing LoVo tumors.

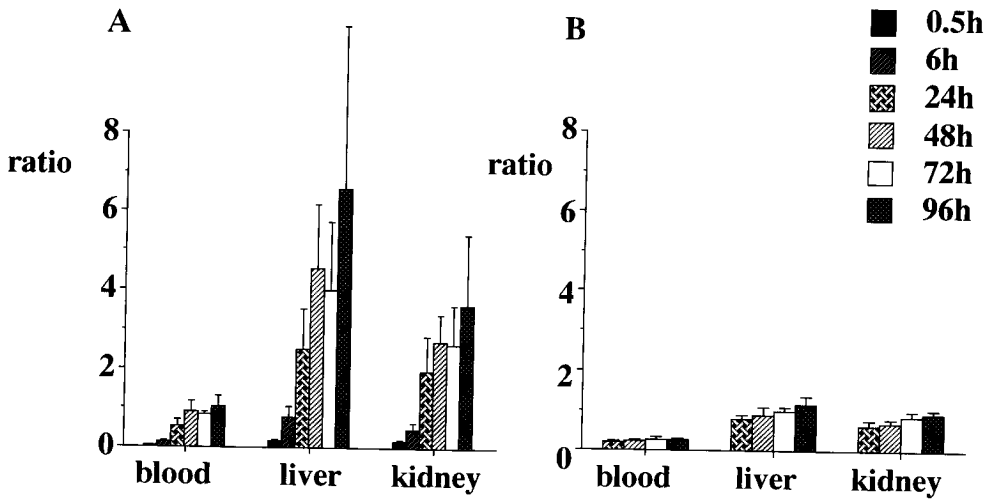

Fig. 5 Tumor-to-organ ratios of ^{125}I-B72.3 (A) and ^{125}I-human IgG (B) in athymic mice bearing LoVo tumors.

Both ^{188}Re-labeled B72.3 and human IgG cleared more quickly from the blood and normal organs than ^{125}I-labeled counterparts (Figures 2 and 3). Tumor-to-organ ratios are shown in Figures 4 and 5. Rapid clearance of ^{188}Re-B72.3 resulted in the high tumor-to-blood ratio of 2.04 ± 0.44 at 96 hours in comparison with 1.05 ± 0.28 for ^{125}I-B72.3, but ^{125}I-B72.3 had higher tumor-to-liver and tumor-to-kidney ratios than ^{188}Re-B72.3.

DISCUSSION

In this study, Mab B72.3 was efficiently labeled with ^{188}Re by a direct technique using reduction of the intrinsic disulfide bonds of the antibody. In addition, Mab B72.3 was prepared for ^{188}Re-labeling and stored as a kit suitable for routine use. Immunoreactivity of ^{188}Re-B72.3 was similar to that of ^{125}I-B72.3.

The *in vitro* cell binding showed specific accumulation of ^{188}Re- and ^{125}I-labeled B72.3 to LoVo cells. In the biodistribution studies, ^{188}Re-B72.3 specifically targeted the LoVo tumors as well as ^{125}I-B72.3. Although absolute tumor accumulation of ^{188}Re-B72.3 was lower than that of ^{125}I-B72.3, rapid clearance of ^{188}Re-B72.3 from the blood resulted in the higher tumor-to-blood ratio. Pimm et al.[20] and Sakahara et al.[21] suggested that the fast blood clearance of reduction-mediated ^{99m}Tc-labeled monoclonal antibodies may be attributed to the release of ^{99m}Tc or small fragments containing ^{99m}Tc from the antibodies. The similar release of ^{188}Re from the reduction-mediated ^{188}Re-labeled B72.3 may explain the rapid blood clearance in our study. Moreover, relatively high liver uptake may be attributable to the metabolism of ^{188}Re-labeled B72.3 in the liver and to the excretion into the biliary system.[21] And the urinary excretion of ^{188}Re-B72.3 me-

tabolites may account for high renal uptake of 3.96 ± 0.48 %ID/g at 24 hours as compared with 3.21 ± 0.81 %ID/g for ^{125}I-B72.3.[21]

Since ^{186}Re is produced by neutron exposure to a ^{185}Re-rich metal target, it is difficult to get high purity of ^{186}Re. On the other hand, ^{188}Re is easily obtained from a ^{188}W/^{188}Re generator without carrier. The mean beta energy levels of ^{188}Re, 0.73 and 0.79 MeV, which represent mean ranges in the soft tissue of 2.7 and 3.1 mm, are an advantage for therapy of tumors which are often heterogeneously necrotic. Although the peak gamma energy of ^{188}Re, 155 keV, is suitable for imaging, Eary et al. suggested that a medium-energy collimator is appropriate to get optimal image resolution and count rate for ^{188}Re because of its higher energy peaks including 478, 633, 829, and 931 keV.[3]

Due to the short half life of ^{188}Re (16.98 hours), a sufficient accumulation is needed to achieve effective irradiation of the target. Techniques aiming for more stable labeling, such as pretreatment of antibodies with 2-iminothiolane in order to provide a thiol reactive position for the attachment rhenium may enable higher tumor accumulation and tumor-to-normal tissue contrast.[22,23]

Another approach, which should improve the stability of rhenium-labeled antibodies, is conjugation of chelating agents. Goldrosen et al. reported that NR-LU-10 Mab, labeled stably with ^{186}Re by using a tetrafluorophenyl-activated ester derivative of the triamide thiolate as a chelate, showed blood, liver, kidney and tumor accumulation of 8.2, 2.3, 1.3, and 9.8%ID/g, respectively, at 24 hours in mice bearing human colon carcinoma.[24] Visser et al. used S-benzoyl-mercaptoacetyltriglycine (*S*-benzoyl-MAG3) for ^{186}Re-labeled E48 F(ab')$_2$ and observed that in mice xenografted with squamous cell carcinoma, 10.4 %ID/g localized in the tumor, with only 2.8 %ID/g in the blood, and less than 1.2%ID/g in the liver and kidney at 48 hours,[25] so that chelate techniques seem efficient in achieving stable rhenium-labeling which enables high tumor-to-nornal tissue ratios. Nevertheless, direct-labeling methods have the advantage of simple kit preparation suitable for clinical studies.

In conclusion, Mab B72.3 was successfully labeled with ^{188}Re by direct labeling and tumor localization was confirmed. Further studies are needed to obtain high tumor-to-normal tissue ratios by direct-labeling methods.

ACKNOWLEDGMENTS

The authors thank the German-Japanese Radiological Affiliation (Osaka, Japan) for the fellowship offered to Masako N. Hosono, and they also thank the Alexander von Humboldt Foundation (Bonn, Germany) for the fellowship awarded to Makoto Hosono.

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