Biodistribution and Dosimetric Study in Medullary Thyroid Cancer Xenograft Using Bispecific Antibody and Iodine-125-Labeled Bivalent Hapten

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The purpose of this study was to evaluate biodistributions and absorbed doses of anti-carcinoembryonic antigen (CEA)/anti-diethylenetriamine pentaacetic acid (DTPA)-indium (anti-DTPA-In) bispecific monoclonal antibody (BsMAb) F6-734 and ^{125}I-labeled DTPA-indium dimer hapten (^{125}I-di-DTPA-In hapten) in athymic mice xenografted with human medullary thyroid cancer. **Methods:** Bispecific monoclonal antibodies F6-679 (anti-CEA/antihistamine) and G7A5-734 (antimelanoma/anti-di-DTPA-In) were used as irrelevant BsMAbs. Athymic mice inoculated with TT medullary thyroid cancer cells expressing CEA were administered BsMAbs F6-734, F6-679 or G7A5-734 and then, 48 hr later, ^{125}I-di-DTPA-In hapten. Iodine-125-labeled F6 F(ab')$_2$ fragment was injected into other groups of mice. Biodistributions were examined at 30 min and 5, 24, 48 and 96 hr after injection of ^{125}I-di-DTPA-In hapten or ^{125}I-labeled F6 F(ab')$_2$. **Results:** In mice injected with BsMAb F6-734 and ^{125}I-di-DTPA-In hapten, tumor uptake was 9.1% ± 2.1%, 8.7% ± 3.5%, 8.0% ± 2.3%, 5.1% ± 0.9% and 3.5% ± 1.5% of the injected dose/g at 30 min and 5, 24, 48 and 96 hr, and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were 37.0 ± 12.5, 32.3 ± 10.9 and 10.4 ± 2.7 at 24 hr. Iodine-125-F6 F(ab')$_2$ fragment showed a tumor uptake of 7.39% injected dose/g and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of 1.8 ± 0.6, 7.3 ± 2.9 and 3.6 ± 1.6 at 24 hr. In mice injected with F6-679 or G7A5-734, tumor uptake and tumor-to-normal tissue ratios were much lower than in the mice injected with F6-734. These results were confirmed by autoradiographic studies that demonstrated clear tumor-to-normal tissue contrast. **Conclusion:** This two-step targeting method seems very potent for the diagnosis and therapy of human medullary thyroid cancer and other CEA-producing tumors because it combines high tumor uptake and low normal tissue background.

Key Words: medullary thyroid carcinoma; anti-carcinoembryonic antigen; anti-diethylenetriamine pentaacetic acid-indium bispecific antibody; pretargeting

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Medullary thyroid cancer (MTC) has a tendency to metastasize to the cervical and mediastinal lymph nodes, lung, liver and bone. Postoperative fibrosis and a wide variety of tumor extents often limit the diagnostic accuracy of ultrasonography, x-ray CT and MRI (*1,2*). Scintigraphic detection with radiolabeled anti-carcinoembryonic antigen (CEA) monoclonal antibody (MAb) has reportedly been useful for evaluating MTC lesions (*3,4*).

In immunoscintigraphy, tumor-to-normal tissue ratios are often only moderate with direct labeling techniques. To date, pretargeting or multistep methods have been developed to improve tumor-to-normal tissue contrast, and some avidin-biotin techniques have been tried for the detection of small metastases as well as primary lesions (*5–7*). Two-step radioimmunotargeting using anti-CEA/anti-diethylenetriamine pentaacetic acid (DTPA)-indium (anti-DTPA-In) bispecific MAb (BsMAb) and di-DTPA-tyrosyl-lysine bivalent hapten (*8,9*) has been proposed for efficient detection of colon carcinoma (*10,11*), MTC (*12,13*), small cell lung carcinoma (*13*) and non–small cell lung carcinoma (*14*).

Although the two-step technique has been evaluated in patients with MTC, its therapeutic potential for MTC has not been investigated in animal models. In this study, we evaluated the potential of the two-step technique by comparing the results of biodistribution and dosimetry with those obtained by the one-step technique and the two-step technique using irrelevant BsMAbs in a mouse model. Absorbed doses in tumors and normal tissues were also determined to calculate tumor-to-normal tissue irradiation ratios.

MATERIALS AND METHODS

Cells and Mice

The MTC cell line TT, derived from a fine needle biopsy specimen of a female and expressing CEA and calcitonin, was obtained from American Type Culture Collection (Manassas, VA). TT cells were cultured in RPMI 1640 culture medium (Life Technologies, Grand Island, NY) supplemented with 1 m*M* glutamine and 10% fetal calf serum.

TT cells were implanted by subcutaneous inoculation of minced tumor into the flanks of 5- to 7-wk-old female BALB/c *nu/nu* mice. Xenografted mice were used when the tumor reached ~0.3 g.

TT xenografts were histopathologically examined with hematoxylin-eosin staining and immunohistochemically using rabbit antihuman CEA (Dakopatts, Glostrup, Denmark).

Antibodies and DTPA-Indium Dimer Hapten

Monoclonal antibody 734 is a murine IgG1, lambda chain antibody (Ab) specific for DTPA-In complexes (*9*). MAb F6 is a murine IgG1, kappa chain Ab specific for human CEA (*9*). Monoclonal antibody G7A5 is a murine IgG1, kappa chain Ab recognizing the human high molecular weight melanoma-associated antigen (*15*) and MAb 679 is a murine IgG1, kappa chain Ab recognizing the structure of histamine-succinyl-glycine (*16*).

Anti-CEA/anti-DTPA-In BsMAb, designated F6-734 (F6-734 BsMAb), was provided by Immunotech (Marseille, France). In brief, this BsMAb was obtained by coupling an equimolecular quantity of a Fab' fragment of an anti-CEA MAb (F6) to a Fab fragment of an anti-DTPA-In MAb (734) previously activated by o-phenylene-dimaleimide.

The bivalent DTPA hapten N-α-DTPA-tyrosyl-n-ε-DTPA-lysine (di-DTPA) was obtained by reaction of DTPA dianhydride with tyrosyl-lysine diacetate (*8*).

F6-734 BsMAb and F6 F(ab')$_2$ were labeled with ^{125}I using the

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chloramine-T method. BsMAbs (40 μg) in 0.3 *M* phosphate buffer (pH 7.5) and ^{125}I (11.1 MBq) for protein labeling were mixed with 2.5 μg chloramine-T (Aldrich, St. Quentin Fallavier, France) dissolved in 0.3 *M* phosphate buffer. After 5 min, the radiolabeled MAb was separated from free radioiodine by Sephadex G-25 gel chromatography (Pharmacia, Uppsala, Sweden). The specific activity for ^{125}I-labeled F6-734 and F6 F(ab')$_2$ was 222 MBq/mg.

Before labeling DTPA-In bivalent hapten with ^{125}I, hapten was saturated with indium in indium chloride solution because MAb 734 only recognizes the structure of the DTPA-In complex. Then, 20 μl di-DTPA-In (0.1 m*M*) were added to 55 MBq ^{125}I and 10 μl chloramine-T, 1 mg/ml in phosphate-buffered saline (0.1 *M*, pH 6). The solution was mixed and incubated for 2 min at room temperature. Then, 10 μl sodium disulfide (Aldrich) in phosphate-buffered saline (0.1 *M*, pH 6) was added and swirled for 5 min. The radioiodinated hapten was separated from free radioiodine by Sep-Pak C_{18} chromatography (Millipore, Bedford, MA). The specific activity of ^{125}I-labeled hapten was 600 μCi/nmol (22.2 MBq/nmol).

Biodistribution of Iodine-125-Labeled F6-734

Increasing doses, 40 μg (0.4 nmol), 80 μg (0.8 nmol) and 160 μg (1.6 nmol), of ^{125}I-labeled F6-734 were administered intravenously to mice xenografted with TT cells (four mice per dose point). At 48 hr after injection, the mice were killed, their tumors and organs were removed and weighed, and radioactivity was counted by a gamma counter. The results were expressed as the percentage of the injected dose/g of tissue (%ID/g). The tumor weight was 0.22 ± 0.11 g for the 12 studied mice, and there was no significant difference among the three groups of mice.

Biodistribution of One- and Two-Step Targeting

Xenografted mice were given 0.5 nmol (50 μg) BsMAbs F6-734, F6-679 or G7A5-734 through the tail vein. After an interval of 48 hr, 40 pmol (52 ng) unlabeled di-DTPA-In and 185 kBq (5 μCi) corresponding to 10 pmol radioiodinated-di-DTPA-In were injected intravenously.

After the injection of ^{125}I-di-DTPA-In hapten, mice were killed at 30 min and 5, 24, 48 and 96 hr (four mice per time point), tumor and organs were removed and weighed and then the radioactivity was counted by a gamma counter.

Other groups of mice were given 37 kBq ^{125}I-labeled F6 F(ab')$_2$. The Ab dose was adjusted to 5 μg/mouse by the addition of unlabeled Ab. At 30 min and 5, 24, 48 and 96 hr, biodistributions were determined (four mice per time point).

The results were expressed as %ID/g. The tumor weight was 0.34 ± 0.20 g for the 80 studied mice, and there was no significant difference among the groups of mice.

Autoradiography

Mice given BsMAbs F6-734 or G7A5-734 plus ^{125}I-di-DTPA-In hapten or ^{125}I-labeled F6 F(ab)'2 were killed at 5 and 24 hr and then embedded in a hemicellulose block with a calibration range of ^{125}I. The block was frozen, and 30-μm-thick sections of the whole animal and the calibration range were made using a cryomacrotome (Cryomacrocut, Leica, Deerfield, IL). The sections were mounted on transparent tape and placed directly on radiographic film for 5 days at −20°C.

Urinary and Fecal Recovery

Four mice inoculated with TT tumors were injected with 0.5 nmol (50 μg) BsMAbs F6-734 through the tail vein and, after an interval of 48 hr, with 40 pmol (52 ng) unlabeled di-DTPA-In and 10 pmol (185 kBq, 5 μCi) ^{125}I-labeled-di-DTPA-In. The mice were housed in a metabolic cage, and urine and stool were collected daily for 4 days, and then radioactivity was counted using a gamma counter (*17*). The recovery was expressed as a percentage of the injected radioactivity.

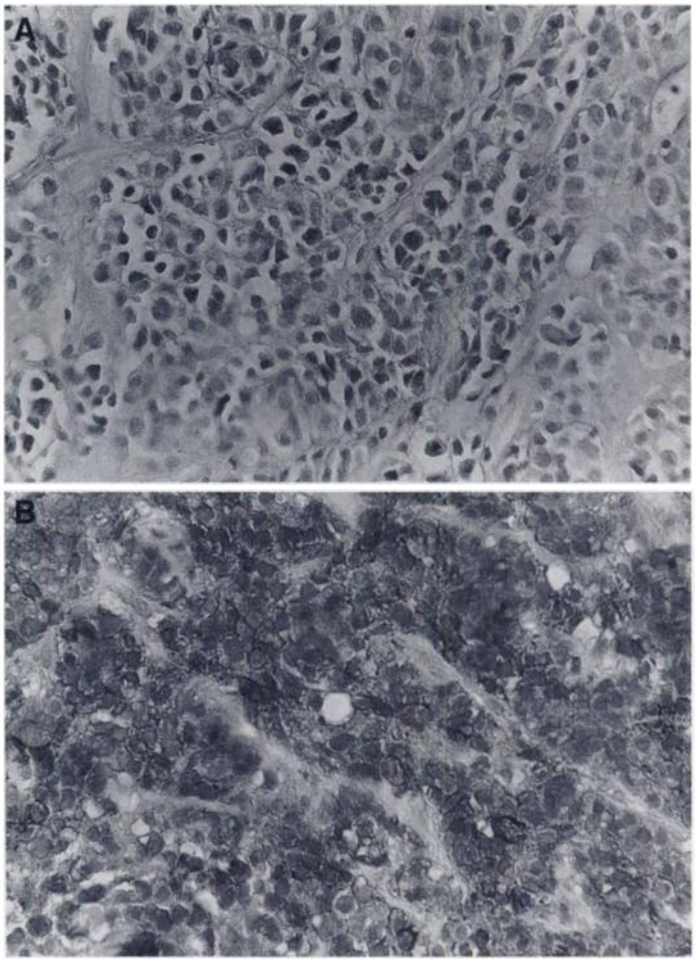

FIGURE 1. Histological studies of TT xenograft (original magnification, ×100). (A) Hematoxylin-eosin staining. Tumor has solid, trabecular structure, with large cells having high nucleo-to-cytoplasmic ratio. (B) Immunohistochemistry using anti-CEA antibody. Strong CEA expression on both surface membrane and cytoplasm.

Dosimetry

To evaluate therapeutic potential, absorbed doses in mice of ^{131}I-labeled di-DTPA-In hapten or MAb were calculated on the basis of the biodistribution data of ^{125}I-labeled di-DTPA-In hapten or MAb (*18,19*). An integrated single exponential curve was fit for the time-activity curve using computer software. The calculation was based on the method in the MIRD pamphlet (*20*). Only beta-particle irradiation was considered, because the mean range of the beta particle represents 95% deposition within 0.99 mm, and gamma emission passed through the mice with little absorption (*19*). Although bone marrow accumulation was not assessed in the biodistribution study, bone marrow irradiation doses were assumed to be 37% of blood doses, as reported by Buchegger et al. (*21*). Whole-body dosimetry was performed for F6-734 plus di-DTPA-In hapten on the assumption that injected radioactivity with subtraction of urinary and fecal excretion had a homogeneous distribution throughout the body.

RESULTS

Figure 1 presents micrographs of a TT xenograft with hematoxylin-eosin staining and immunohistochemistry using an anti-CEA Ab. It showed a solid, trabecular structure with large cells having a high nucleo-to-cytoplasmic ratio. Immunohistochemistry demonstrated strong CEA expression on surface membrane and cytoplasm.

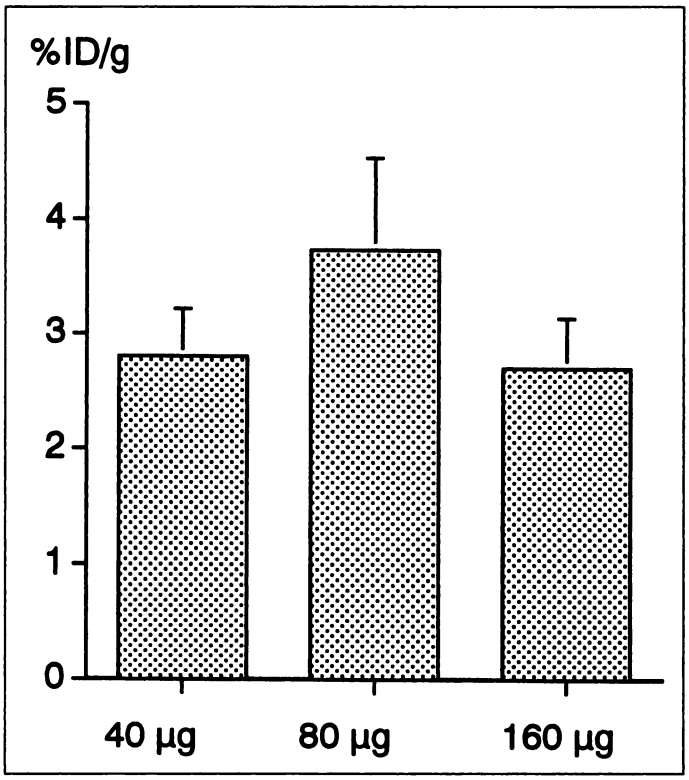

FIGURE 2. Tumor uptake in mice given 40, 80 and 160 µg of ^{125}I-labeled F6-734 BsMAb. Almost constant uptake is observed in this range of antibody dose.

In athymic mice given 40, 80 and 160 µg ^{125}I-labeled BsMAb F6-734, a substantial amount of radioactivity was observed in TT xenografts (Fig. 2). Tumor uptake was not significantly different in this Ab dose range.

Tumor uptake of F6-734 plus ^{125}I-di-DTPA-In hapten, ^{125}I-labeled F6 F(ab')$_2$, F6-679 plus ^{125}I-di-DTPA-In hapten and G7A5-734 plus ^{125}I-di-DTPA-In hapten is illustrated in Figure 3. F6-734 plus ^{125}I-di-DTPA-In hapten localized in the tumor quickly, showing an uptake of 9.1 ± 2.1%ID/g at 30 min. The tumor uptake of ^{125}I-labeled F6 F(ab')$_2$ reached 7.4 ± 0.9%ID/g at 24 hr. Only low tumor uptake was observed for F6-679 plus ^{125}I-di-DTPA-In hapten and G7A5-734 plus ^{125}I-di-DTPA-In hapten, and the latter localized in the tumor more than the former.

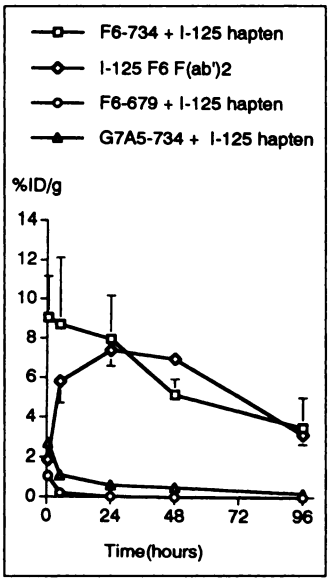

FIGURE 3. Tumor uptake for one- and two-step methods in xenografted mice. Combination of anti-CEA/anti-DTPA-In BsMAb F6-734 and ^{125}I-di-DTPA-In hapten indicated rapid and elevated tumor localization compared with directly labeled F6 F(ab')$_2$. BsMAbs F6-679 (anti-CEA/antihistamine) and G7A5-734 (antimelanoma/anti-di-DTPA-In) were used as irrelevant BsMAbs.

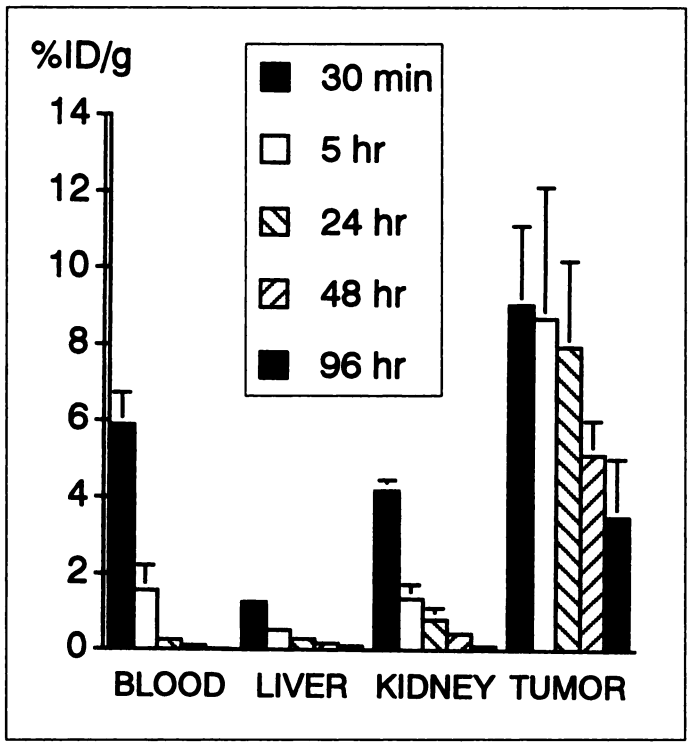

FIGURE 4. Biodistribution of BsMAb F6-734 and ^{125}I-di-DTPA-In hapten in blood, liver, kidney and tumor at 30 min and 5, 24, 48 and 96 hr.

Tissue distribution of F6-734 plus ^{125}I-di-DTPA-In hapten is demonstrated in Figure 4. Radioactivity cleared quickly from the blood. The kidney showed higher accumulation than other normal organs.

Tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of F6-734 plus ^{125}I-di-DTPA-In hapten, ^{125}I-labeled F6 F(ab')$_2$, F6-679 plus ^{125}I-di-DTPA-In hapten and G7A5-734 plus ^{125}I-di-DTPA-In hapten are illustrated in Figure 5. For F6-734 plus ^{125}I-di-DTPA-In hapten, the tumor-to-blood ratios were 1.6 ± 0.3, 5.8 ± 1.0, 37.0 ± 12.5, 66.3 ± 36.0 and 181.8 ± 78.1 at 30 min and 5, 24, 48 and 96 hr, respectively, and tumor-to-liver and tumor-to-kidney ratios were 32.3 ± 10.9 and 10.4 ± 2.7 at 24 hr. For ^{125}I-labeled F6 F(ab')$_2$, tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were 1.8 ± 0.6, 7.3 ± 2.9 and 3.6 ± 1.6, respectively, at 24 hr.

In mice injected with F6-734 plus ^{125}I-di-DTPA-In hapten, cumulative recovery of injected radioactivity in urine and stool of mice is shown in Figure 6. Up to Day 4, 76.9 ± 10.7% and 3.67 ± 1.5% of the total injected radioactivity were excreted in urine and stool, respectively.

Absorbed radiation doses of ^{131}I-labeled di-DTPA-In hapten or MAb, calculated on the basis of the biodistribution data of ^{125}I-labeled counterparts, and effective half-times of radioactivity in the tumor, liver, kidney, lung, blood, bone marrow and whole body are presented in Table 1. For F6-734 plus ^{131}I-di-DTPA-In hapten, absorbed doses were 5.5, 0.15 and 0.31 cGy/µCi in the tumor, liver and kidney, respectively, whereas ^{125}I-labeled F6 F(ab')$_2$ showed 6.8, 0.39 and 0.69 cGy/µCi in the tumor, liver and kidney, respectively.

Autoradiography (Fig. 7) demonstrated clear visualization of xenografts in mice for both one- and two-step methods. However, the two-step method showed a higher tumor-to-normal tissue contrast than the one-step method at 5 and 24 hr after injection of hapten.

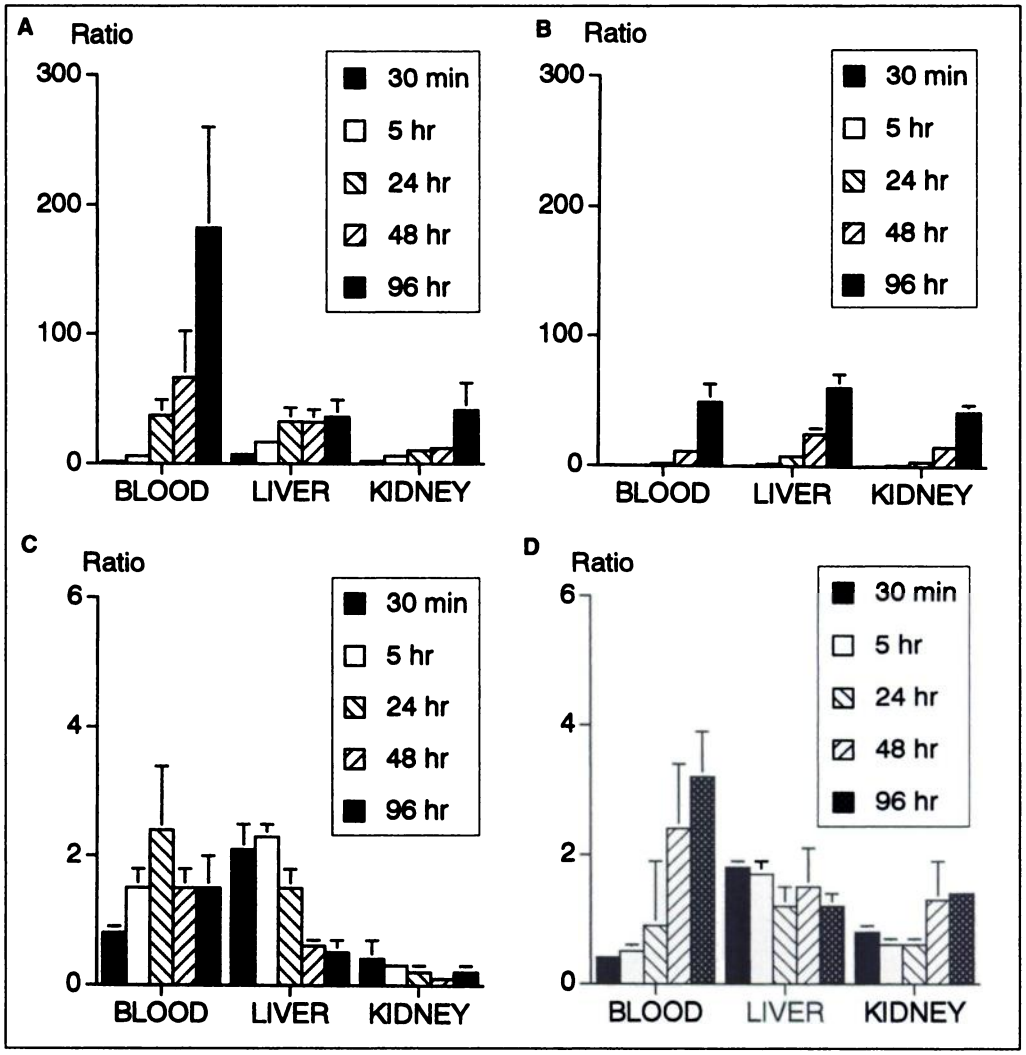

FIGURE 5. Tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of BsMAb F6-734 plus ^{125}I-di-DTPA-In hapten (A), ^{125}I-labeled F6 F(ab')$_2$ (B), BsMAb F6-679 (anti-CEA/antihistamine) plus ^{125}I-di-DTPA-In hapten (C) and G7A5-734 (antimelanoma/anti-di-DTPA-In) plus ^{125}I-di-DTPA-In hapten (D) at 30 min and 5, 24, 48 and 96 hr.

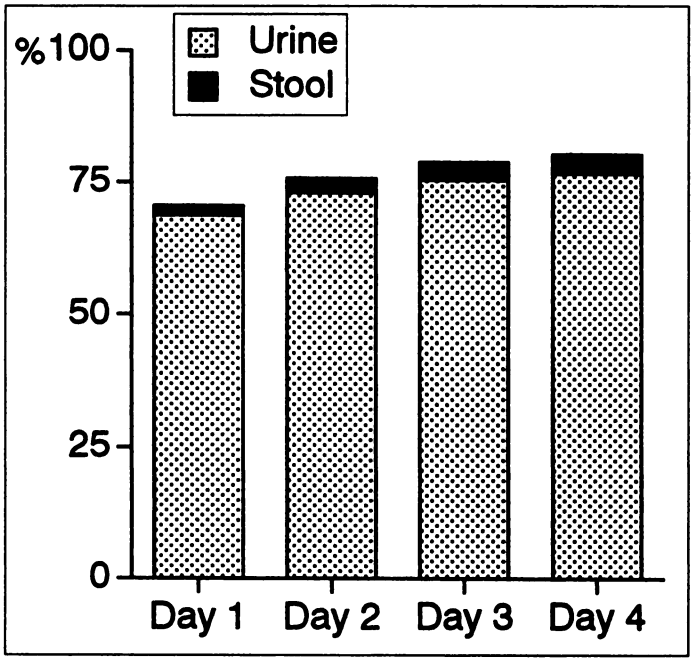

FIGURE 6. Recovery of radioactivity in urine and stool of mice administered BsMAb F6-734 plus ^{125}I-di-DTPA-In hapten. Up to Day 4, 76.9 ± 10.7% and 3.67 ± 1.5% of total injected radioactivity was excreted in urine and stool.

DISCUSSION

Because CEA is present in most MTC tumors (*22,23*), CEA is thought to be a suitable target for Ab-guided imaging and radiation therapy. Promising results have been obtained in patients with MTC using directly labeled anti-CEA MAbs (*3,4,13*).

In this study, the capability of anti-CEA/anti-DTPA-In BsMAb and ^{125}I-di-DTPA-In in targeting MTC was confirmed in a xenografted mouse model. Biodistribution, excretion of radioactivity in urine and stool and absorbed dose were quantified. Images were obtained by autoradiographic studies, which demonstrated higher tumor-to-normal tissue contrast with specific two-step targeting than with one-step and nonspecific two-step systems.

Iodine-125-labeled F6-734 BsMAb showed similar percentages of injected radioactivity in tumors at MAb doses of 40, 80 and 160 μg. Therefore, we adopted 50 μg of BsMAb, which is in the range between 40 and 80 μg, for the two-step method.

The combination of BsMAb F6-734 and ^{125}I-di-DTPA-In hapten demonstrated tumor-to-blood, tumor-to-liver and tumor-to-kidney accumulation ratios of 182, 36 and 42 at 96 hr after injection of hapten compared with 49, 60 and 55, respectively, using a ^{125}I-labeled F6 F(ab')$_2$ fragment.

Also, maximum uptake level was 9.06 ± 2.10%ID/g for the two-step method and 7.39 ± 0.86%ID/g for the one-step method. Therefore, it is clearly indicated that the pretargeting technique combines high tumor uptake and high tumor-to-normal tissue ratios.

TABLE 1
Absorbed Doses in Tumor and Normal Tissues for Iodine-131 Calculated on the Basis of Iodine-125 Biodistribution Data

Organ	F6-734 + hapten	F6 F(ab')$_2$	F6-679 + hapten	G7A5-734 + hapten
Tumor				
Dose	5.50	6.80	0.03	0.35
T_{eff}	100.3	150.5	15.1	37.6
Liver				
Dose	0.15	0.39	0.01	0.27
Ratio	36.7	17.4	1.9	1.3
T_{eff}	37.6	15.1	23.2	50.2
Kidney				
Dose	0.31	0.69	0.11	0.35
Ratio	17.1	9.9	0.2	1.0
T_{eff}	23.2	13.7	21.5	25.1
Lung				
Dose	0.14	0.67	0.02	0.18
Ratio	39.9	10.2	1.4	2.0
T_{eff}	16.7	14.3	16.7	23.2
Blood				
Dose	0.18	1.48	0.03	0.32
Ratio	30.6	4.6	1.0	1.1
T_{eff}	13.7	12.0	15.1	16.7
Bone marrow				
Dose	0.07	0.55	0.01	0.12
Ratio	78.6	12.4	2.7	3.0
T_{eff}	nd	nd	nd	nd
Whole body				
Dose	0.16	nd	nd	nd
Ratio	34.4	nd	nd	nd
T_{eff}	21.5	nd	nd	nd

Dose = absorbed dose (cGy/μCi); T_{eff} = effective half-time (hr); Ratio = tumor-to-normal tissue ratio of absorbed doses; nd = not done.

BsMAbs F6-679 (anti-CEA/antihistamine) and G7A5-734 (antimelanoma/anti-DTPA-In hapten) were used as nonspecific BsMAbs in this study. Because DTPA hapten does not bind to BsMAb F6-679, it is rapidly eliminated through the urinary system, which leads to low accumulation in the tumor and normal organs. Meanwhile, BsMAb G7A5-734 is thought to have nonspecific distributions in the tumor and normal organs and to trap radiolabeled DTPA hapten, which accounts for higher accumulation of radioactivity than the combination F6-679 and DTPA hapten. These results suggest that the system of relevant BsMAb and hapten is highly specific for tumors expressing CEA.

On the assumption that ^{131}I-di-DTPA-In hapten and MAb have the same biodistribution as ^{125}I-di-DTPA-In hapten and MAb, absorbed doses in mice for ^{131}I were calculated on the basis of the biodistribution data of ^{125}I. Tumor-to-normal tissue ratios of absorbed dose were significantly higher in the two-step method than in the one-step method using directly radioiodinated F(ab')$_2$ (Table 1). For absorbed dose, the tumor-to-liver ratios were ~37 and ~17, and tumor-to-kidney ratios were ~17 and ~10 for the two- and one-step methods, respectively. Peltier et al. (*12*) reported results of the two-step imaging in which tumor-to-liver and tumor-to-kidney absorption ratios were ~10 to ~110 and ~2 to ~50, respectively, in five patients with MTC on the basis of a dosimetric imaging study, but the ratios varied greatly, probably because of inhomogeneity of tumor size and antigen expression in cancer patients. This study confirms that the two-step technique enables elevated tumor-to-normal tissue contrast.

In this study, the specific two-step method delivered 5.5 cGy/μCi to the tumor. Thus, 1 mCi hapten would give 55 Gy to the tumor, whereas 1.5 and 3.1 Gy would be absorbed in the liver and kidney. This pretargeting technique showed the potential of not only detection but also internal radiation therapy. A higher tumor-to-kidney ratio of this two-step method would be an advantage even if the kidney would be a dose-limiting organ in

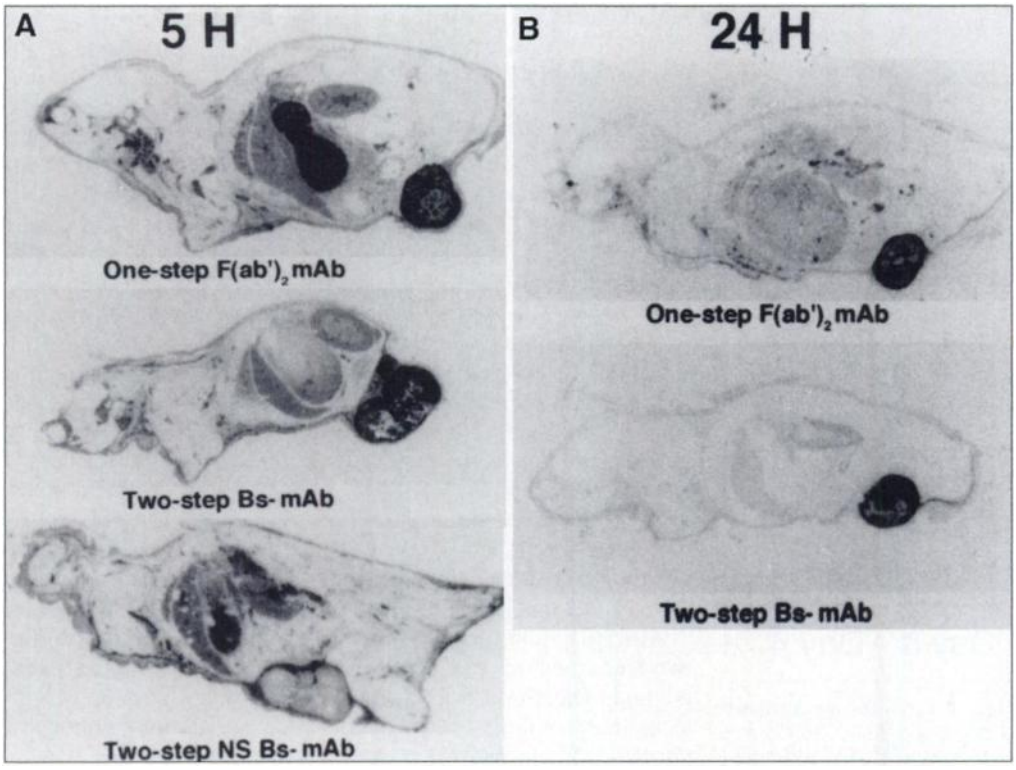

FIGURE 7. Autoradiographs of xenografted mice at (A) 5 hr and (B) 24 hr. Tumors are clearly visualized with both ^{125}I-labeled F6 F(ab')$_2$ and BsMAb F6-734 plus ^{125}I-di-DTPA-In hapten. Normal tissues are much less dense in the two-step than the one-step method. One-step F(ab')$_2$ mAb, ^{125}I-labeled F6 F(ab')$_2$; two-step BsMAb, BsMAb F6-734 plus ^{125}I-di-DTPA-In hapten; two-step NS BsMAb, G7A5-734 (antimelanoma/anti-di-DTPA-In) plus ^{125}I-di-DTPA-In.

radioimmunotherapy. Moreover, this targeting technique could be theoretically applicable to most CEA-producing tumors.

Some pretargeting methods use the avidin-biotin system characterized by four identical avidin subunits, each bearing a single binding site for biotin (*5,7*). Avidin, egg white protein, is strongly immunogenic toward humans. Our pretargeting with BsMAb and DTPA hapten, free from avidin, seems more suitable for repeated scintigraphy and therapeutic application (*11*).

CONCLUSION

The two-step pretargeting method allows us to obtain tumor-to-normal tissue contrast that is higher than the one-step method, which suggests that this technique has strong therapeutic potential.

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