Two-Step Targeting and Dosimetry for Small Cell Lung Cancer Xenograft with Anti-NCAM/Antihistamine Bispecific Antibody and Radioiodinated Bivalent Hapten

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The "affinity enhancement system," a two-step targeting technique using bispecific antibody and radiolabeled bivalent hapten, has been reported to be useful for carcinoembryonic antigen-expressing tumors. The purpose of this study was to evaluate the efficacy of this method for targeting human small cell lung cancer using an antineural cell adhesion molecule antibody. **Methods:** Antineural cell adhesion molecule/antihistamine bispecific antibody NK1NBL1–679 was prepared by coupling an equimolecular quantity of a Fab' fragment of NK1NBL1 to a Fab fragment of antihistamine 679. Athymic mice inoculated with NCI-H69 small cell lung cancer cells expressing neural cell adhesion molecule were administered bispecific antibody and then 48 h later ^{125}I-labeled bivalent histamine hapten. ^{125}I-labeled intact NK1NBL1 was injected into other groups of mice. Biodistributions were examined as a function of time. **Results:** In mice of the two-step targeting, tumor uptake was 2.5 ± 0.2, 3.2 ± 0.4, 6.4 ± 2.0, 7.2 ± 2.7, 6.1 ± 2.1 and 2.2 ± 0.4 %ID/g at 5, 30 min, 5, 24, 48 and 96 h, and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were 1.4 ± 1.1, 10.8 ± 13.2 and 4.6 ± 4.7, respectively, at 5 h, whereas ^{125}I-labeled NK1NBL1 showed a tumor uptake of 5.7 ± 0.4 %ID/g and tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of 0.3 ± 0.1, 1.1 ± 0.2 and 0.9 ± 0.1, respectively, at 5 h. These results were confirmed by autoradiographic studies, which demonstrated clear tumor-to-normal tissue contrast. Dosimetry showed that the affinity enhancement system could enhance the therapeutic potential of the antineural cell adhesion molecule antibody NK1NBL1. **Conclusion:** This two-step targeting method seems promising for the diagnosis and therapy of small cell lung cancer.

Key Words: small cell lung carcinoma; antineural cell adhesion molecule/antihistamine bispecific antibody; pretargeting; affinity enhancement system

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Bispecific monoclonal antibody (BsAb) containing antitumor and antihapten binding sites and radiolabeled bivalent hapten have been proposed for a novel method of pretargeted or multistep immunoscintigraphy, which should reduce background activity in the blood and normal tissues and prolong activity residence time in the tumor (*1,2*). This two-step technique has been designated the "affinity enhancement system" (AES). In the AES, BsAb is administered to target tumor cells, and then radiolabeled bivalent hapten is injected after a delay, allowing excess BsAb to clear from the circulation. In addition, the bivalence of the hapten accounts for a higher affinity of the hapten for cell-bound than for free-circulating BsAb.

Several researchers (*2–5*) reported results of immunoscintigraphy in patients with colorectal, medullary thyroid and non–small cell lung cancers by this AES using anticarcinoembryonic antigen (CEA)/antidiethylenetriamine pentaacetic acid (DTPA) BsAb and hapten consisting of two DTPA moieties linked by a dipeptide bond.

Small cell lung cancer (SCLC), characterized by endocrine features, a tendency to metastasize and high chemo- and radiosensitivity, represents 20%–25% of lung cancers, and the mortality rate of patients with SCLC remains >90% at 2 y after diagnosis (*6*). Because most post-therapy patients have eventual recurrence, and because tumors become refractory to repeated therapy, antibody-guided internal radiotherapy has been expected as a further therapeutic modality. Several reports (*7–10*) have described animal studies of SCLC therapy using radiolabeled monoclonal antibodies (MAbs) reactive with some SCLC-related antigens. Neural cell adhesion molecule (NCAM), expressed in normal human tissues such as nerves, endocrine glands and natural killer (NK) cells (*11*), is considered the most specific of the SCLC-related antigens. Because most SCLC tumors express NCAM on the surface membrane of the cells (*11*), it is thought to be an optimal target for radioimmunodetection and radioimmunotherapy.

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Radioimmunoscintigraphy by a pretargeting or two-step technique for SCLC has been tried using anti-CEA/anti-DTPA BsAb and bivalent DTPA-indium hapten (*12*). Because CEA is strongly present in only <35% of SCLC (*13*), another method of the AES with anti-NCAM/antihapten BsAb may have an advantage of higher incidence of antigen expression in SCLC tumors. The purpose of this study was to evaluate the potential of the anti-NCAM antibody-guided AES technique for localizing SCLC lesions in a mouse model by conducting dosimetry.

MATERIALS AND METHODS

Monoclonal Antibody and Radiolabeling

The murine MAb NK1NBL1 (IgG1) (*14*), reacting with human NCAM, was supplied by Immunotech (Marseille, France). The MAb 679 is a murine IgG1, κ-chain antibody recognizing the structure of histamine-succinyl-glycine (*15*). The MAb OC125, used as a nonspecific Ab in this study, was generated by immunizing mice with human ovarian serous cystadenocarcinoma and recognized a high-molecular weight glycoprotein CA125 (*16*). Anti-NCAM/antihistamine BsAb designated as NK1NBL1–679 (NK1NBL1–679 BsAb) was produced as previously reported (*1,2*). In brief, this BsAb was obtained by coupling an equimolecular quantity of a Fab′ fragment of an anti-NCAM MAb (NK1NBL1) to a Fab fragment of an antihistamine (679) activated beforehand by o-phenylene-dimaleimide.

The bivalent histamine hapten Nα-acetyl-Nε-(histamine-succinyl-glycyl)-lysyl-tyrosyl-Nε-(histamine-succinyl-glycyl)-lysinamide (di-HSG-Lys-Try-Lys) (mol wt 980) was obtained by solid-phase peptide synthesis (*15,17*). To label bivalent histamine hapten with ^{125}I, 20 μL hapten (0.1 mmol/L) were added to 55 MBq ^{125}I and 10 μL chloramine-T (Aldrich, St. Quentin Fallavier, France), 1 mg/mL in phosphate-buffered saline (PBS) 0.1 mol/L pH 6. The solution was mixed and incubated 2 min at room temperature. Then, 10 μL sodium disulfide (Aldrich) in PBS 0.1 mol/L pH 6 were 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).

The intact MAbs NK1NBL1 and OC125 were labeled with ^{125}I using the chloramine-T method. MAbs (40 μg) in 0.3 mol/L phosphate buffer (PB), pH 7.5, and ^{125}I (11.1 MBq) for protein labeling were mixed with 2.5 μg chloramine-T dissolved in 0.3 mol/L PB. 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 NK1NBL1 and OC125 was 210–282 MBq/mg.

Cell Line and Xenografts

The SCLC cell line NCI-H69 cells (*18*), obtained from the American Type Culture Collection (Manassas, VA), were cultured in RPMI 1640 culture medium (Life Technologies, Grand Island, NY) supplemented with 1mmol/L glutamine and 10% fetal calf serum. For the studies in mice, NCI-H69 SCLC cells were implanted by subcutaneous inoculation of a tumor mince into the flanks of 5- to 7-wk-old female BALB/c nu/nu mice.

Biodistribution of One-Step and Two-Step Targeting

Xenografted mice were administered 0.5 nmol (50 μg) BsAb NK1NBL1–679 through the tail vein. After an interval of 48 h, 185 kBq (5 μCi) corresponding to 10 pmol (10 ng) radioiodinated bivalent histamine hapten were intravenously injected into the mice. Mice were killed at 5, 30 min, 5, 24, 48 or 96 h (4 mice/time point), tumor and organs were removed and weighed and the radioactivity was counted by a gamma counter. Other groups of mice were administered 111 kBq (3 μCi) ^{125}I-labeled NK1NBL1 or OC125, and biodistribution was determined at 1, 5, 24, 48 and 96 h, and at 168 h for ^{125}I-labeled NK1NBL1.

The results are expressed as the percentage of the injected dose per gram of tissue (%ID/g). The tumor weight was 0.56 ± 0.28 g (n = 68), and there was no significant difference among the groups.

Autoradiography

Xenografted mice were intravenously injected with 111 kBq (3 μCi) ^{125}I-labeled NK1NBL1. Other xenografted mice were administered BsAb NK1NBL1–679 and 48 h later 370 kBq (10 μCi) ^{125}I-labeled histamine hapten. The mice were killed at 24 or 48 h and embedded in a hemicellulose block, with a calibration range of ^{125}I. The block was then frozen, and 30-μm-thick sections of the

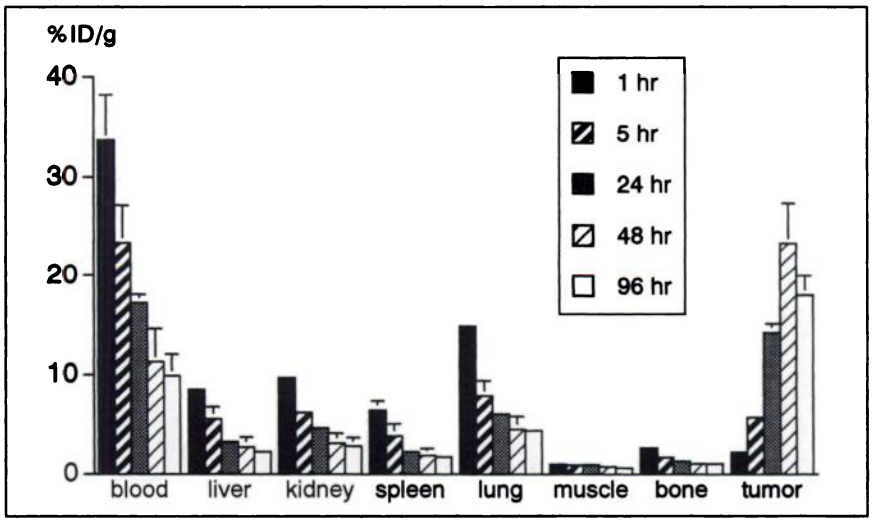

FIGURE 1. Biodistribution of one-step method with ^{125}I-labeled anti-NCAM antibody NK1NBL1 in mice inoculated with NCI-H69 tumor.

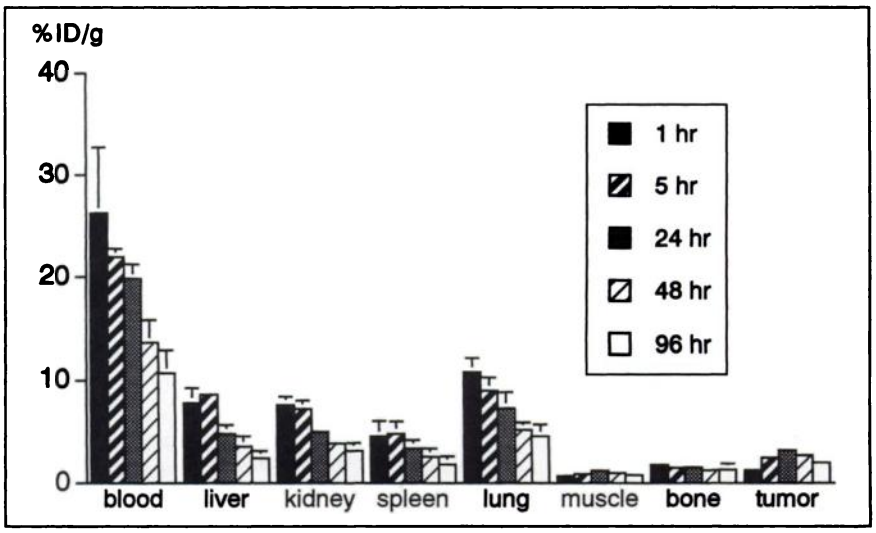

FIGURE 2. Biodistribution of one-step method with ^{125}I-labeled anti-CA125 antibody OC125, nonspecific to NCAM, in mice inoculated with NCI-H69 tumor.

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 d at −20°C.

Dosimetry

To evaluate therapeutic potential, absorbed doses in mice for ^{131}I-labeled histamine hapten or MAb were calculated on the basis of the biodistribution data of ^{125}I-labeled histamine hapten or MAb (*9,10,19*). An integrated single exponential curve was fit for the time activity using the computer software CA-Cricket Graph III (Computer Associates, San Diego, CA). The calculation was based on the method in the Medical Internal Radiation Dose pamphlet (*20*). A uniform distribution of radioactivity was assumed in the organs and tumor. Only β-particle irradiation of ^{131}I (nonpenetrating component) was considered because the mean range of the β particle represents 95% deposition within 0.99 mm, and gamma emission passed through mice with only a little absorption (*10*). The energy imparted to organs other than the source was considered too small to be of significance in the dose calculation. 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*).

RESULTS

In mice of the one-step targeting, tumor uptake was 2.2 ± 0.2, 5.7 ± 0.4, 14.3 ± 0.9, 23.4 ± 4.0, 18.1 ± 2.0 and 6.8 ± 2.2 %ID/g at 1, 5, 24, 48, 96 and 168 h for ^{125}I-labeled NK1NBL1 (Fig. 1) and 1.2 ± 0.2, 2.5 ± 0.3, 3.1 ± 0.1, 2.7 ± 0.2 and 1.9 ± 0.4 %ID/g at 1, 5, 24, 48 and 96 h for ^{125}I-labeled OC125 (Fig. 2), nonspecific to NCAM, respectively, suggesting that ^{125}I-labeled NK1NBL1 had specific binding to NCI-H69 xenografts in vivo.

On the other hand, in mice of the two-step technique with BsAb NK1NBL1–679 + ^{125}I-labeled bivalent histamine hapten, tumor uptake was 2.5 ± 0.2, 3.2 ± 0.4, 6.3 ± 2.0, 7.2 ± 2.7, 6.1 ± 2.1 and 2.2 ± 0.4 %ID/g at 5, 30 min, 5, 24, 48 and 96 h (Fig. 3), which was lower than in one-step targeting. ^{125}I-labeled NK1NBL1 showed tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios of 0.3 ± 0.1,

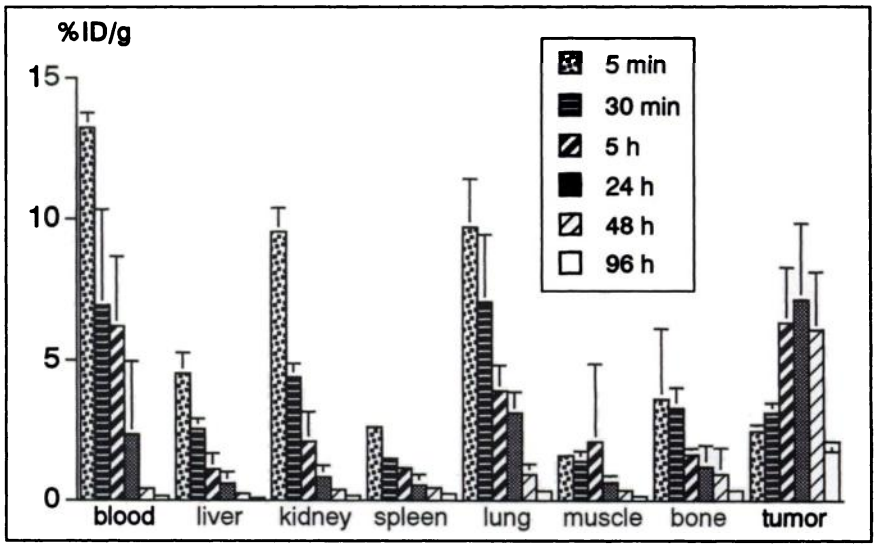

FIGURE 3. Biodistribution of two-step method with anti-NCAM/antihistamine hapten BsAb NK1NBL1–679 and ^{125}I-labeled bivalent histamine hapten in mice inoculated with NCI-H69 tumor.

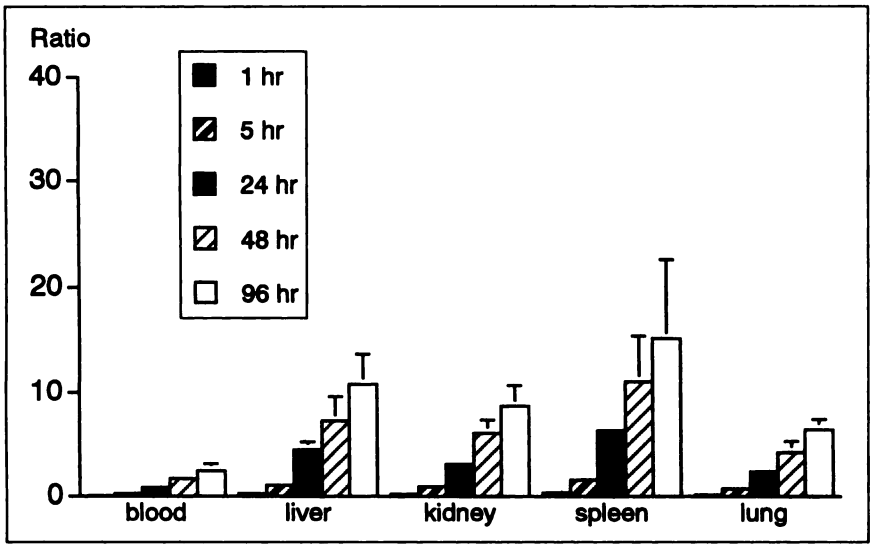

FIGURE 4. Tumor-to-normal tissue ratios of one-step method with ^{125}I-labeled anti-NCAM antibody NK1NBL1 in mice inoculated with NCI-H69 tumor.

1.1 ± 0.2 and 0.9 ± 0.1 at 5 h (Fig. 4). In the two-step method, tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios were 1.4 ± 1.1, 10.8 ± 13.2 and 4.6 ± 4.7 at 5 h (Fig. 5), and the tumor-to-blood ratio reached 15.4 ± 5.1 at 48 h. These results were confirmed by autoradiographic studies, which demonstrated clear tumor-to-normal tissue contrast (Fig. 6).

Dosimetric calculation indicated tumor irradiation of 3.42 cGy/μCi for the two-step method versus 8.86 cGy/μCi for the one-step method (Table 1). Tumor-to-liver, tumor-to-kidney, tumor-to-lung, tumor-to-blood and tumor-to-bone marrow ratios of absorbed doses were 14.3, 8.6, 5.3, 6.2 and 16.8 versus 4.2, 3.5, 2.6, 1.0 and 2.6, for the two-step and one-step methods, respectively.

DISCUSSION

The AES targeting technique with anti-CEA/anti-DTPA hapten and ^{111}In-labeled or radioiodinated bivalent DTPA hapten has been developed and successfully used for the diagnosis of colorectal cancer, medullary thyroid cancer and nonSCLC (*2–5*). In this study, another method of the AES with anti-NCAM/antihistamine BsAb NK1NBL1–679 and ^{125}I-labeled bivalent histamine hapten substantially improved tumor-to-normal tissue ratios in absolute accumulation levels as well as absorbed doses. Because NCAM is abundantly expressed in most SCLC, it is an optimal target of radioimmunotherapy for SCLC. A drawback is that as a member of the immunoglobulin superfamily, it mediates cell-cell interactions and appears in characteristic spatiotemporal patterns during development (*22*) and is expressed in normal tissues, including neural tissues, endocrine glands and NK cells.

The one-step method of radioiodinated intact anti-NCAM MAb NK1NBL1 could deliver 8.9, 2.1, 2.5 and 3.4 cGy/μCi to the tumor, liver, kidney and lung, respectively, which were comparable with the results of another radioiodinated intact anti-NCAM MAb NE150 of 7.7, 1.4, 1.7 and 2.6 cGy/μCi (*10*). In the latter study of the radioiodinated

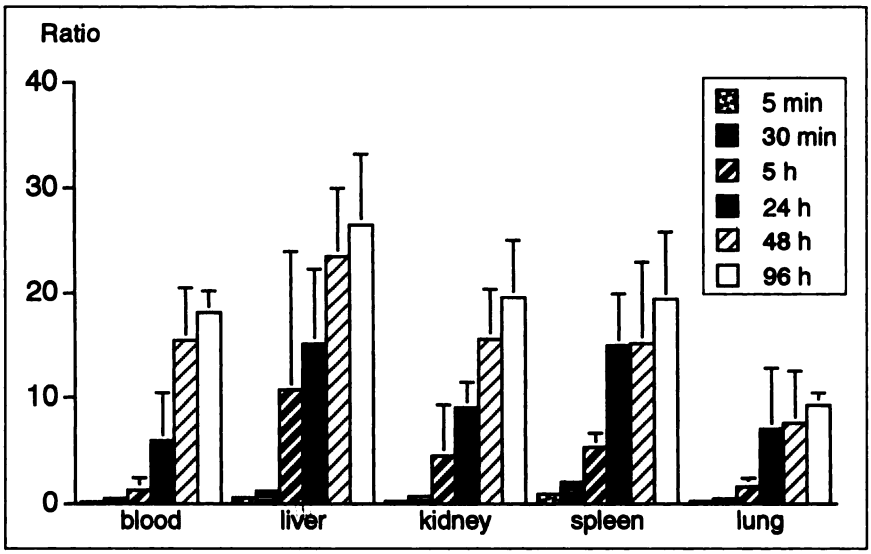

FIGURE 5. Tumor-to-normal tissue ratios of two-step method with anti-NCAM/antihistamine hapten BsAb NK1NBL1–679 and ^{125}I-labeled bivalent histamine hapten in mice inoculated with NCI-H69 tumor.

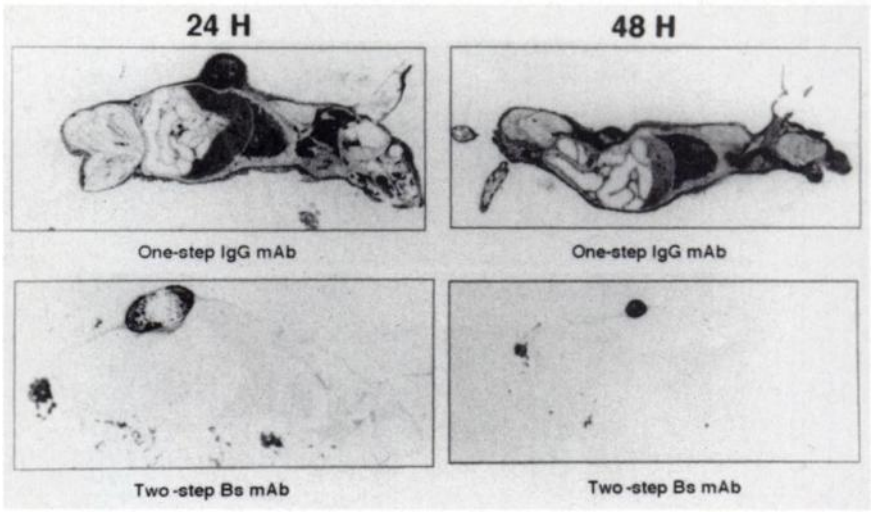

FIGURE 6. Autoradiographs of mice xenografted with NCI-H69 tumor by one-step (upper) and two-step (lower) methods at 24 and 48 h. For one-step method, ^{125}I-labeled NK1NBL1 was intravenously injected into mice. For two-step method, BsAb NK1NBL1–679 and, 48 h later, ^{125}I-labeled bivalent histamine hapten were injected into mice.

anti-NCAM MAb NE150, a significant regression of SCLC xenografts was observed in mice given 300 μCi ^{131}I-labeled NE150. Therefore, it is thought that the MAb NK1NBL1 also has therapeutic potential.

Moreover, the two-step method enhanced the therapeutic efficacy of this MAb, as indicated by the following tumor-to-liver, tumor-to-kidney, tumor-to-lung, tumor-to-blood and tumor-to-bone marrow ratios of absorbed doses of 14.3, 8.6, 5.3, 6.2 and 16.8, respectively, versus 4.2, 3.5, 2.6, 1.0 and 2.6, respectively, in the one-step method. On the other hand, in a previous mouse model of medullary thyroid cancer conducted by the two-step method with anti-CEA/anti-DTPA BsAb and radioiodinated bivalent DTPA hapten, dosimetry showed that tumor-to-liver, tumor-to-blood and tumor-to-bone marrow ratios were 36.5, 30.6 and 78.6, respectively, for BsAb + radioiodinated bivalent DTPA hapten versus 17.4, 4.6 and 12.4, respectively, for a one-step method with directly radioiodinated anti-CEA F(ab')2 (*23*). In this study with anti-NCAM antibody, these tumor-to-normal tissue ratios were lower than in the previous study with anti-CEA antibody for both one- and two-step methods. This may be attributable in part to the lower affinity between anti-NCAM MAb NK1NBL1 and NCAM of $K_a = 5.2 \times 10^7$ M^{-1} versus $K_a = 2.1 \times 10^9$ M^{-1} between anti-CEA MAb F6 and CEA (data not given). Nevertheless, the two-step technique has substantially improved the tumor-to-normal tissue ratios compared with the one-step technique.

TABLE 1
Absorbed Doses in Tumor and Normal Tissues for ^{131}I Calculated on Basis of ^{125}I Biodistribution Data

	NK1NBL1–679+ Hapten			NK1NBL1		
Organ	Dose	Ratio	Teff	Dose	Ratio	Teff
Tumor	3.42	—	100.3	8.86	—	75.3
Liver	0.24	14.3	37.6	2.13	4.2	60.2
Kidney	0.42	8.6	18.8	2.52	3.5	60.2
Lung	0.65	5.3	20.1	3.40	2.6	60.2
Blood	0.55	6.2	15.8	9.21	1.0	12.0
Bone marrow	0.20	16.8	ND	3.40	2.6	ND

Dose = absorbed dose (cGy/μCi); Ratio = tumor-to-normal tissue ratio of absorbed doses; Teff = effective half-time (h); ND = not done.

A problem concerning the dosimetry in this study is that the distribution of radiolabeled MAbs in mice administered a therapeutic dose of MAb may differ from that with a tracer dose of MAb because of rapid tumor growth of SCLC xenografts and tumor damage as a result of therapy (*24*). Dosimetry with a therapeutic dose of MAb should be performed in further examinations.

In previous studies with BsAbs and haptens (*2–4,12,23*), the combination of the anti-CEA F6/anti-DTPA 734 BsAb and bivalent DTPA hapten frequently has been used. In this study, we used the system of an anti-NCAM/antihistamine BsAb and a bivalent histamine hapten. The antihistamine Ab 679 has an affinity constant of $K_a = 6.8 \times 10^9$ M^{-1} to its antigen (*15,17*), whereas anti-DTPA 734 has an affinity constant of $K_a = 7.7 \times 10^9$ M^{-1} to its antigen (*1*). Although the affinity is slightly lower, the antihistamine/histamine system of this study has an advantage of potential coupling of hapten with radioactive rhenium such as ^{186}Re and ^{188}Re through chelate on the glycine-lysine.

Ornadel et al. (*25*) reported that SCLC tumors were not clearly targeted using ^{131}I-labeled anti-NCAM MAb, probably due to distribution of the antibody to normal tissues, including NK cells. Also, normal tissues that express NCAM on their cell surface membrane but are not protected by the blood-brain barrier, such as the peripheral nerve and adrenal and thyroid glands, may be susceptible to the NCAM MAbs. Our two-step system may reduce the background accumulation of anti-NCAM MAb and enable clear visualization and effective irradiation of targets, because it can minimize accumulation in normal tissues, in which the antigen density is relatively low (*1,2*).

For the pretargeting techniques, internalization of antigen-antibody complex can be an obstacle, because it disturbs

access of the radiolabeled molecule to antigen-bound antibody. Kwa et al. (*26*) reported that only one ^{125}I-labeled anti-NCAM MAb out of three was internalized by 22.3% in NCI-H69 cells whereas the other two were not, and internalization and subsequent dehalogenation did not prevent the intracellular accumulation of tumor radioactivity. In this study, internalization was not quantified. However, in our particular pretargeting method, internalization did not interfere much with localization of SCLC lesions, because a good tumor accumulation and a sufficient tumor-to-normal tissue contrast were achieved by our pretargeting technique.

CONCLUSION

The two-step method with anti-NCAM/antihistamine BsAb and bivalent histamine hapten enabled higher tumor-to-normal tissue ratios than the one-step method. Thus, it could enhance the therapeutic potential of the anti-NCAM MAb NK1NBL1.

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