Pretargeting of Carcinoembryonic Antigen–Expressing Tumors with a Biologically Produced Bispecific Anticarcinoembryonic Antigen × Anti-Indium–Labeled Diethylenetriaminepentaacetic Acid Antibody

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Abstract

Purpose: The aim of these studies was to develop a pretargeting strategy for CEA-expressing cancers using biologically produced bispecific monoclonal antibodies (bsMAb). The bsMAbs used in this system have affinity for the carcinoembryonic antigen on the one hand, and for indium-labeled diethylenetriaminepentaacetic acid (DTPA), on the other.

Experimental Design: Stable quadroma clones producing bsMAb MN-14xDTIn-1 were isolated. LS174T tumor–bearing mice were injected with 1 to 100 μg of bsMAb followed by 1 to 60 ng of an ^{111}In-labeled bivalent peptide [Ac-Phe-Lys(DTPA)-Tyr-Lys(DTPA)-NH_2]. Mice were killed at 24 hours postinjection and the biodistribution of the radiolabel was determined. The biodistribution of diDTPA labeled with four different radionuclides (^{111}In, ^{99m}Tc, nonresidualizing ^{125}I, and residualizing ^{125}I) was determined at various time points postinjection following pretargeting of LS174T tumors with bsMAb MN-14xDTIn-1.

Results: Optimal tumor targeting was observed when tumors were pretargeted with 10 μg of bsMAb MN-14xDTIn-1 and when 6 ng of a radiolabeled peptide was given 72 hours later. The uptake of the four radiolabels in LS174T tumors at 4 hours postinjection was similar. However, at later time points, the ^{111}In-label and residualizing ^{125}I-label were better retained in the tumor than the nonresidualizing ^{125}I label. Although the absolute uptake in the tumor (in terms of percentage of injected dose per gram of tissue) was 5-fold lower than the uptake obtained with directly labeled MN-14, the pretargeting strategy revealed much higher tumor-to-blood ratios due to the rapid clearance of the radiolabel from the circulation as compared with ^{111}In-MN-14 (445 ± 90 and 5.3 ± 1.1, respectively, at 72 hours postinjection).

Conclusions: Effective targeting of carcinoembryonic antigen-expressing tumors was achieved with a newly produced bispecific antibody. The ^{111}In-labeled L-amino acid peptide and ^{125}I-D-amino acid peptide were better retained in the tumor than the ^{99m}Tc- and ^{125}I-L-amino acid peptide. Very high tumor-to-blood ratios were obtained due to rapid background clearance.

Colorectal cancer is a malignancy with a relatively high incidence worldwide (15% of all malignancies in both genders; ref. 1). Radioimmunotherapy (RIT) with radiolabeled antibodies directed against carcinoembryonic antigen (CEA) has shown encouraging results in preclinical studies and in patients with small volume disease, comparable to established chemotherapy (2–5). Anti-CEA monoclonal antibodies (MAb) were used in the majority of RIT studies with colorectal cancer patients. CEA is expressed at high levels in colon adenocarcinoma cells (6) and in medullary thyroid carcinoma cells (7). In addition, CEA is expressed at lower levels in various normal tissues, including the colon, stomach, esophagus, tongue, cervix, and prostate (8).

RIT with directly labeled MAbs was effective for the treatment of hematologic tumors most likely due to their relative radiosensitivity and their easy accessibility. For diagnostic purposes of solid tumors, radioimmunoscintigraphy revealed adequate images within 72 hours. However, RIT with directly labeled MAbs cannot deliver therapeutically effective radiation doses to solid tumors. The relatively long circulation half-life of the radiolabeled MAbs limits the activity dose that can be administered without causing severe damage to normal tissues, especially the bone marrow (9–11).

Goodwin and coworkers (12) were the first to propose that separation of the targeting agent (MAb) and the effector agent (radioactivity) might be advantageous in RIT. Two main

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pretargeting approaches have been investigated intensively: (*a*) based on the avid interaction between biotin and (strept)avidin, and (*b*) based on the use of bispecific antibodies (bsMAb). In the studies described here, a bsMAb is used that has a high affinity for CEA and for indium-labeled diethylenetriaminepentaacetic acid (bsMAb/anti-CEA × anti-DTPA(In)). Following i.v. administration of the bsMAb, the bsMAb accumulates in the tumor and clears from the circulation. In the second step, a radiolabeled bivalent peptide (diDTPA) is administered that is trapped in the tumor by the bsMAbs or cleared very rapidly from the circulation, thereby reducing the radiation burden to normal cells (13) and improving the tumor-to-blood (T/B) ratio (14).

In the second step of the approach, a radiolabeled bivalent peptide (diDTPA(In)) is administered. As compared with the use of a monovalent radiolabeled hapten, the uptake and retention of the radiolabel in the tumor improved when a bivalent peptide was used. It is thought that this improvement is caused by the so-called affinity enhancement system: a bivalent peptide could cause the formation of a bridge between two adjacent bsMAbs at the tumor cell surface.

We have developed and stabilized a quadroma clone, producing a bispecific anti-CEA × anti-DTPA(In) antibody (bsMAb MN-14xDTIn-1) to pretarget CEA-expressing tumors. After pretargeting the colorectal tumor with the bsMAb MN-14xDTIn-1, pretargeted tumors can be targeted with a radiolabeled diDTPA-peptide.

In addition to the L-amino acid peptide allowing the use of In-111, a peptidase-resistant peptide consisting of D-amino acids labeled with I-125 was used in the studies to enhance the residence time of the iodine label in the tumor (15). In these studies, we tested and optimized the pretargeting strategy with CEA-expressing tumors based on the newly developed bsMAb MN-14xDTIn-1. At optimized conditions, the pretargeting strategy was tested with diDTPA peptides labeled with various radionuclides (^{111}In, ^{99m}Tc, nonresidualizing ^{125}I, and residualizing ^{125}I).

Materials and Methods

LS174T colorectal carcinoma

The human colorectal cancer cell line LS174T was obtained from the American Type Culture Collection (Rockville, MD). LS174T is a rapidly growing, moderately to well-differentiated human colorectal carcinoma cell line, expressing moderate levels of CEA (5,000-10,000 epitopes/cell; ref. 16). Cells were grown in RPMI medium, supplemented with FCS and penicillin/streptomycin (Life Technologies, Grand Island, NY). Before s.c. inoculation of mice to establish LS174T xenografts, tumor cells were washed with 0.9% NaCl, disaggregated with 0.25% trypsin and resuspended in complete medium to the appropriate concentration (4.5×10^5 cells/200 μL) and injected s.c. (200 μL/mouse).

Production and purification of the bsMAb MN-14xDTIn-1

Two hybridoma cell lines were fused: MAb MN-14 (anti-CEA) and MAb DTIn-1 (anti–indium-labeled DTPI, anti-DTPA(In)), essentially as described previously (17). The murine MN-14 MAb is a high-affinity anti-CEA MAb ($K_a = 10^9$ mol/L), kindly provided by Immunomedics, Inc. (Morris Plains, NJ; ref. 18). Preceding cell fusion, the MN-14 cell line was made thymidine kinase–deficient by culturing the cells in the presence of escalating concentrations of bromodeoxyuridine (Boehringer Mannheim, Mannheim, Germany). The production and characterization of MAb DTIn-1 (IgG_{2a}) has been described elsewhere (17). Preceding cell fusion, the DTIn-1 cell line was made hypoxanthine-guanine phosphoribosyltransferase–deficient by culturing the cells in the presence of escalating doses of 8-aza-guanine (Sigma-Aldrich, Zwijndrecht, the Netherlands). After cell fusion, quadroma cells were selected in hypoxanthine, aminopterin, thymidine medium. Isolated bsMAb MN-14xDTIn-1–producing cells were stabilized as described previously (17). The selected stable quadroma clone was expanded in Integra CL 1000 culture flasks (IBS Integra Biosciences, Micronic BV, the Netherlands). The quadroma cells produce the heavy and light chains of both parental antibodies, which are randomly combined (19). To purify the bsMAb from the supernatant, protein-A chromatography (Econo-Pac, protein A cartridge, Bio-Rad, Hercules, CA) was done as described previously (17). Following each purification step, eluted fractions were tested for the bispecific antibody activity in a double specific ELISA and their bsMAb titers were determined. Briefly, ELISA plates (Falcon, BD Biosciences, San Jose, CA), coated with bovine serum albumin-DTPA(In) (0.42 μg/100 μL), were incubated at 37°C for 1 hour with a serial dilution of collected fractions. Wells were subsequently incubated with a WI-2-biotin solution (25 ng/well), a streptavidin-peroxidase solution, 10 μg/well tetramethylbenzidine solution containing H_2O_2, and finally 2 mol/L H_2SO_4. Following every step, plates were washed extensively with 0.5% bovine serum albumin/PBS. The extinction, measured at 450 nm, was determined by an ELISA microplate reader (model 3550, Bio-Rad microplate reader). IgG was further purified by a cation-exchange chromatography using a mono-S column (Amersham Pharmacia, Roosendaal, the Netherlands) on a biologic chromatography system (Bio-Rad, Hercules, CA; Fig. 1; ref. 17). Finally, the bsMAb was purified on an immuno-affinity column (Amino Link Kit, Perbio Science, Etten-Leur, the Netherlands) loaded with an anti-MN-14 antibody (WI-2; ref. 20). In this final purification step, all the antibody analogues deprived of a CEA binding site were eliminated. Bound material was eluted with gentle elution buffer (Perbio Science). The material was dialyzed against PBS, concentrated to 1 mg/mL, filtered through a 0.2 μm filter and stored at −80°C until use.

Bivalent peptides

L-Amino acid peptide. The characteristics of the L-amino acid peptide (IMP156) were described previously (21). The peptide, Ac-Phe-Lys(DTPA)-Tyr-Lys(DTPA)-NH_2 (diDTPA-FKYK, molecular weight 1,377 Da), was formulated in a lyophilized labeling kit, containing 11 μg bivalent peptide, 50 mg 2-hydroxypropyl-β-cyclodextrin, and 4.4 mg citrate (pH 4.2). The characteristics of the L-amino acid peptide (IMP192) for peptide labeling with ^{99m}Tc, were described previously (21). This tetrapeptide was conjugated with thiosemicarbonylglyoxylcysteinyl to the COOH terminus lysine residue: Ac-Lys(DTPA)-Tyr-Lys(DTPA)-Lys(TscGC)-NH_2 (molecular weight 1,590 g/mol) to allow labeling with ^{99m}Tc/^{188}Re. This peptide was formulated in a lyophilized

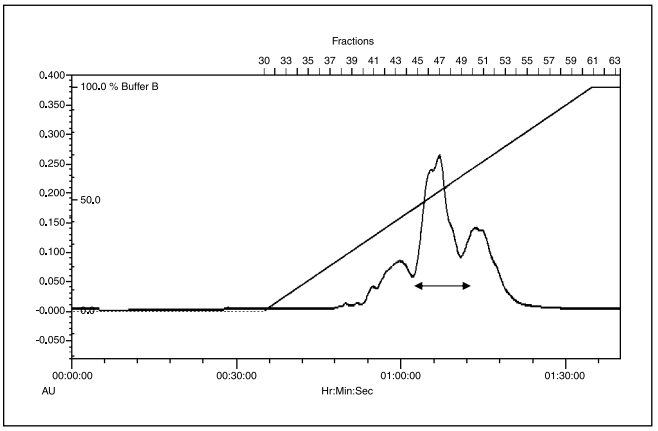

Fig. 1. Purification of the bsMAb MN-14xDTIn-1 using cation-exchange chromatography. Protein A-purified material was eluted from a mono-S column with a 0 to 400 mmol/L LiCl gradient in 40 mmol/L NaAc (pH 5.2). Fractions 44 to 49 (*between the arrows*) contained bsMAb MN-14xDTIn-1 as determined by the double specific ELISA.

labeling kit containing 40 μg peptide, 100 μg $SnCl_2$, 1 mg 2,5-dihydroxybenzoic acid, 10% 2-hydroxypropyl-β-cyclodextrin, 200 mmol/L glucoheptonate, 21 mmol/L acetate (pH 5.3) and six molar equivalents of $InCl_3$.

D-Amino acid peptide. The synthesis and characteristics of this D-amino acid peptide: Ac-DPhe-DLys(DTPA)-DTyr-DLys(DTPA)-NH_2 (diDTPA-fkyk, molecular weight 1,377 Da) were described previously (15).

Radiolabeling

^{111}In-L-amino acid peptide. Eleven micrograms of lyophilized diDTPA-FKYK were reconstituted in 1 mL of saline. To 15 μL of the peptide solution, 65 μL 40 mmol/L NH_4Ac, and 1.5 mCi $^{111}InCl_3$ (Tyco Health Care, Petten, the Netherlands) were added and the reaction mixture was incubated for 60 minutes at room temperature. The radiochemical purity was determined by instant TLC on silica gel strips with methanol/water (55:45) and citrate buffer (pH 6.0) as the mobile phase. When the radiochemical purity exceeded 95%, a 3-fold molar excess $InCl_3$ was added to saturate the DTPA chelates with In^{3+}.

^{125}I-L-amino acid peptide and ^{125}I-D-amino acid peptide. The peptides were iodinated according to the chloramine T method (22). To 200 ng diDTPA, 15 μL chloramine T (1.82 mg/mL) and 0.75 mCi $Na^{125}I$ (Amersham Cygne, Den Bosch, the Netherlands) were added. After 2 minutes of incubation at room temperature, the reaction was stopped by adding 100 μL of sodium metabisulfite (3.37 mg/mL). Subsequently, the pH of the solution was lowered with 1:10 (v/v) 1 mol/L NH_4Ac (pH 5.4) and a 3-fold molar excess In^{3+} was added. The solution was purified with an activated C-18 SepPak cartridge (Waters, Milford, MA). The radiochemical purity was determined by both instant TLC on silica gel strips with citrate buffer (pH 6.0) as mobile phase and by reversed phase high-performance liquid chromatography on an Agilent 1100 series LC system (Agilent Technologies, Palo Alto, CA).

^{99m}Tc-L-amino acid peptide. This diDTPA peptide was labeled using a ^{99m}Tc-labeling kit containing 8 μg of lyophilized diDTPA-KYKK, conjugated with a thiosemicarbonylglyoxylcysteinyl chelate to the COOH terminus Lys residue as described previously (23, 24). This peptide was formulated containing 40 μg bivalent peptide, 100 μg $SnCl_2$, 1 mg 2,5-dihydroxybenzoic acid, 10% 2-hydroxypropyl-β-cyclodextrin, 200 mmol/L glucoheptonate, 21 mmol/L acetate (pH 5.3) and six molar equivalents of $InCl_3$.

MN-14-DTPA-^{111}In. Conjugation of MAb MN-14 with *p*-isothiocyanatobenzyl-DTPA (ITC-DTPA) was done essentially as described by Ruegg et al. (25). Briefly, 1 mL MAb MN-14 (10 mg/mL) was mixed with 110 μL (1.0 mol/L) of $NaHCO_3$ (pH 9.5) and 1.76 mg of ITC-DTPA (50-fold molar excess). Following incubation at room temperature for 1 hour, the reaction mixture was dialyzed overnight in a Slide-A-Lyzer (10 kDa cutoff, Pierce, Rockford, IL) against 50 mmol/L NH_4Ac (pH 5.4). Subsequently, the MN-14-ITC-DTPA conjugate was diluted in 50 mmol/L NH_4Ac to 1 mg/mL, and aliquots were stored at −20°C. Radiolabeling of MN-14-ITC-DTPA: 300 μCi $^{111}InCl_3$ was added to 75 μL of (1 mg/mL) MN-14-ITC-DTPA and incubated at room temperature for 45 minutes. The labeling efficiency of the MAb was determined by instant TLC on silica gel strips with 0.15 mol/L citrate buffer (pH 6.0) as the mobile phase.

MN-14-^{125}I. Iodination of the MAb MN-14 was done according to the iodogen method (26). To a tube coated with 50 μg of iodogen, 10 μL (0.5 mol/L) phosphate buffer (pH 7.4), 75 μL (50 mmol/L) phosphate buffer (pH 7.4), 11.5 μL MN-14 (7.8 mg/mL), and 180 μCi $Na^{125}I$ (Amersham Cygne, Den Bosch, the Netherlands) were added. The mixture was incubated for 10 minutes at room temperature, immediately followed by a purification step on a PD-10 column eluted with PBS and 0.5% bovine serum albumin.

Biodistribution experiments

Female BALB/c *nu/nu* mice, 6 to 9 weeks old, were injected s.c. with a LS174T cell suspension (4.5×10^5 cells/200 μL). When tumors sizes were between 50 and 300 mm^3, the biodistribution experiments were initiated. All reagents were injected i.v. via the tail vein (200 μL).

Groups of four or five animals received escalating doses of bsMAb or peptide to determine the optimal protein and peptide dose. Additionally, the optimal interval between both injections was determined.

At various time points after the injection of the radiolabeled peptide, mice were killed by CO_2 asphyxiation and blood was obtained by heart puncture. Tissues (tumor, muscle, lung, spleen, kidney, liver, and small intestine) were dissected, weighed, and their radioactivity was determined in a gamma counter (Wallac wizard 3″ 1480 automatic gamma counter).

The biodistribution of diDTPA peptides labeled with one of the three radionuclides (In-111, Tc-99m, nonresidualizing I-125, and residualizing I-125) was determined at optimized conditions (10 μg bsMAb, 72-hour intervals, and 6 ng radiolabeled peptide).

As a reference, the targeting of ^{111}In-labeled MAb MN-14 was determined in mice with s.c. LS174T tumors. Mice received 10 μg i.v. (4 μCi/μg) ^{111}In-labeled MAb MN-14 and were dissected at 4, 24, 48, and 72 hours postinjection.

To permit calculation of the radioactive uptake in each organ as a fraction of the injected dose, an aliquot of the injection dose was counted simultaneously. Results were expressed as a percentage of the injected dose per gram of tissue (% ID/g). The studies were approved by the local Animal Welfare Committee and done in accordance with their guidelines.

Scintigraphic imaging

LS174T tumor–bearing mice were pretargeted with 10 μg bsMAb MN-14xDTIn-1, and after 72 hours, mice were injected i.v. with 3.7 MBq (100 μCi) 6 ng ^{111}In-labeled peptide. Mice were anaesthetized with a mixture of enflurane (Ethrane, Abbott BV, Amstelveen, the Netherlands), nitrous oxide and oxygen. Subsequently, the mice were placed prone on a single head gamma camera (Orbiter, Siemens Medical Systems Inc., Hoffman Estates, IL) equipped with a parallel-hole, medium energy collimator. Mice were imaged at 5 minutes, 1, 4, and 24 hours (300,000 counts), at 48 hours (200,000 counts), 72 hours (100,000 counts), and 96 hours (75,000 counts) after injection of the radiolabel.

Statistical analysis

All mean values are given ± SD. Statistical analysis was done using the unpaired *t* test when two groups were analyzed and the one-way ANOVA was used when more than two groups were analyzed. The level of significance was set at $P < 0.05$.

Results

Purification of the bispecific monoclonal antibody MN-14xDTIn-1. Supernatants of the cultured quadroma cells were extensively purified. Following protein-A chromatography, bsMAb-containing fractions were collected (IgG concentration of 140.4 ng/mL still gave a positive signal in the bispecific ELISA) and purified with cation exchange chromatography (Fig. 1). Fractions 44 to 49 were bsMAb-positive, as determined by the double specific ELISA, and collected (IgG concentration of 35.2 ng/mL still gave a positive signal in the bispecific ELISA). Finally, the bsMAb-containing solution was purified using anti-CEA affinity chromatography (IgG concentration of 2.2 ng/mL still gave a positive signal in the bispecific ELISA). The observed bsMAb titer reduction is an indication for the purification of the solution, correlating with the increment of the bsMAb MN-14xDTIn-1 concentration of the solution.

Bispecific monoclonal antibody dose-finding study. The results of the bsMAb MN-14xDTIn-1 dose-finding study are depicted in Fig. 2. LS174T tumor–bearing mice were pretargeted with escalating protein doses (1-100 μg) of the bsMAb MN-14xDTIn-1, followed by administration of 6 ng of the ^{111}In-labeled L-amino acid peptide (IMP156) 72 hours later. Uptake of the

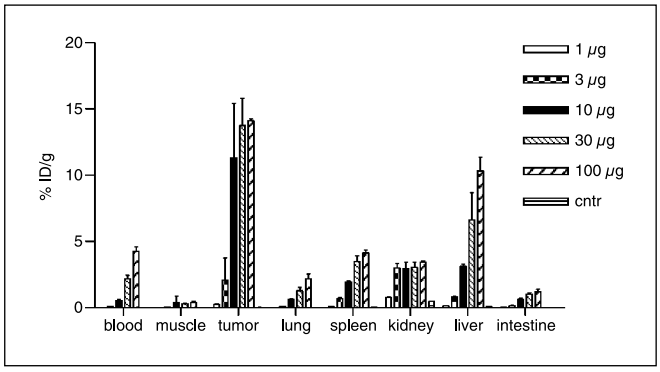

Fig. 2. Biodistribution of the ^{111}In-label in the bsMAb MN-14xDTIn-1 dose escalation study. LS174T tumor – bearing mice were pretargeted with an increasing dose of bsMAb, and 72 hours later, mice received 6 ng of ^{111}In-labeled peptide. Control mice were only injected with 6 ng of ^{111}In-labeled peptide. Mice were killed at 24 hours postinjection, the uptake was expressed as % ID/g (average tumor size, 0.62 g).

radiolabel in the tumor increased significantly by enhancing the bsMAb dose from 1 to 3 to 10 μg (0.25 ± 0.05% ID/g, 2.1 ± 1.7% ID/g, and 11.3 ± 4.1% ID/g at 24 hours postinjection, respectively). At higher protein doses (>10 μg bsMAb), the uptake of the radiolabel in the tumor stabilized, whereas the radioactivity concentration in the circulation and the liver increased. Highest T/B ratios were obtained with 3 and 10 μg bsMAb (51.5 ± 3.5 and 45.1 ± 0.9, respectively). As a control group, tumor-bearing mice received 6 ng of the radiolabeled peptide. The uptake of the radiolabel in the non – pretargeted tumor (<0.1% ID/g at 24 hours postinjection) was significantly lower as in the pretargeted tumors, confirming the specificity of the pretargeting procedure. In this study, the radioactivity in the liver increased at higher bsMAb doses. This was most likely due to the enhanced levels of peptide-bsMAb complexes in the circulation observed at enhanced bsMAb doses. In the LS174T pretargeting system, 10 μg of bsMAb MN-14xDTIn-1 was selected as the optimal bsMAb dose.

Peptide dose-finding study. Figure 3 shows the results of the ^{111}In-labeled peptide dose escalation study. LS174T tumors were pretargeted with 10 μg bsMAb MN-14xDTIn-1 and 72 hours later, mice were injected with various doses of ^{111}In-labeled L-amino acid peptide (IMP156, 0.6-60 ng). The uptake of the radiolabel in the tumor was similar at peptide doses between 0.6 and 20 ng (20.0 ± 1.4% ID/g and 16.38 ± 2.2% ID/g at 24 hours postinjection, respectively). When mice received 60 ng of the ^{111}In-labeled peptide, the uptake in the tumor was significantly lower (9.9 ± 1.0% ID/g). Due to lower blood levels (expressed as % ID/g) at increasing peptide doses, the T/B ratios increased from 50.1 ± 9.4 at 0.6 ng to 162 ± 42 at 60 ng peptide (from 6 to 60 ng peptide, the T/B ratios were similar). The absolute uptake of the peptide in the tumor was calculated at each peptide dose (0.12 ng peptide/g tumor at 0.6 ng peptide to 5.9 ng peptide/g tumor at 60 ng peptide). In subsequent experiments, a radiolabeled bivalent peptide dose of 6 ng was used.

Interval between the bispecific monoclonal antibody and peptide administrations. The results of the experiment to determine the optimal interval between bsMAb/peptide injections are summarized in Fig. 4. Tumors were pretargeted with 10 μg bsMAb MN-14xDTIn-1 and 1, 3, or 7 days later mice received 6 ng of the ^{111}In-labeled L-amino acid peptide (IMP156). No significant differences in uptake of the radiolabel in the tumor were observed at these three intervals (1 day, 10.9 ± 0.8% ID/g; 3 days, 11.4 ± 4.9% ID/g; and 7 days, 7.2 ± 2.9% ID/g at 24 hours postinjection). By increasing the interval between both injections, the radioactivity concentration in the circulation and in the normal organs decreased, with the exception of the kidneys. An interval of 1 day provided a relatively low T/B ratio (71.8 ± 16.5 at 24 hours postinjection). When a longer interval was used, higher T/B ratios were obtained: 180 ± 36 at 3 days and 241 ± 58 at 7 days. In subsequent experiments, an interval of 3 days was used.

Biodistribution of ^{111}In-, ^{99m}Tc-, nonresidualizing ^{125}I-, and residualizing ^{125}I-labeled bivalent peptide. Tumor-bearing mice were pretargeted with 10 μg bsMAb MN-14xDTIn-1 and 3 days later mice were injected with 6 ng of one of the four radiolabeled peptides. The biodistribution of the radiolabel was determined at various time points postinjection (Fig. 5). At 4 hours postinjection of the radiolabeled peptide, the uptake of all peptides in the tumor was in the same order of magnitude. With time, the radiolabel cleared from the tumor irrespective of the radiolabel used. The iodinated L-amino acid peptide (Fig. 5C) cleared from the tumor much faster than the other radiolabeled peptides; tumor retention of ^{125}I-L-amino acid from 24 hours postinjection onwards was significantly lower than that of the other three radiolabels. In general, the uptake of the ^{99m}Tc-labeled peptide in the circulation and in the normal organs (Fig. 5B) was significantly higher than that obtained with the other three radiolabeled peptides. The ^{111}In-label (Fig. 5A) and ^{125}I-D-amino acid peptide (Fig. 5D) showed the best retention in the tumor (4.4 ± 1.3% ID/g and 4.9 ± 1.1% ID/g at 72 hours postinjection, respectively). In Fig. 6, images of LS174T tumor – bearing mice targeted with the ^{111}In-label at optimized conditions [10 μg bsMAb MN-14xDTIn-1, 72-hour interval, and 6 ng (3.7 MBq) ^{111}In-IMP156] are shown. From 1 to 4 hours postinjection, distinct accumulation of the radiolabel in the tumor was observed.

Biodistribution of ^{111}In-labeled MN-14. LS174T tumor – bearing mice were injected i.v. with 10 μg ^{111}In-labeled MN-14 and the biodistribution of the radiolabel was determined at 4, 24, 48, and 72 hours postinjection (Fig. 7). The uptake of the radiolabel in the tumor increased significantly from 4 to

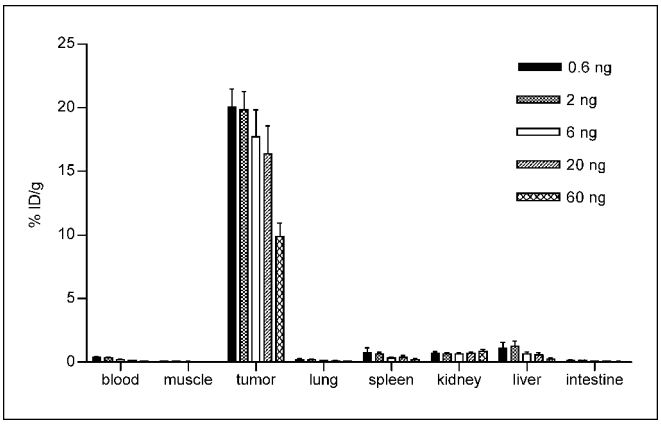

Fig. 3. ^{111}In-labeled peptide dose escalation study. LS174T tumor – bearing mice were pretargeted with 10 μg of bsMAb MN-14xDTIn-1, and 72 hours later, an increasing dose of ^{111}In-labeled peptide was administered. Mice were killed at 24 hours postinjection, the uptake was expressed as % ID/g (average tumor size, 0.13 g).

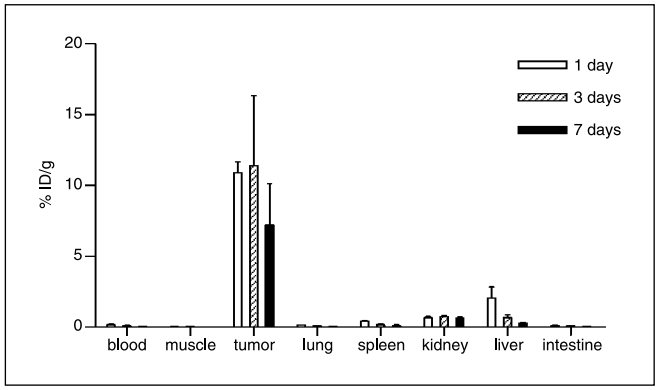

Fig. 4. The influence of the interval between both administrations of CEA-expressing tumors. LS174T tumor – bearing mice were pretargeted with 10 μg of bsMAb MN-14xDTIn-1, and 1, 3, or 7 days later, mice were injected with 6 ng of ^{111}In-labeled peptide. Mice were killed at 24 hours postinjection, the uptake was expressed as % ID/g (average tumor size, 0.35 g).

24 hours postinjection (13.1 ± 5.1% ID/g and 49.0 ± 10.2% ID/g, respectively). From 24 to 72 hours postinjection, uptake of the radiolabel in the tumor did not change significantly. The T/B ratios were relatively low: from 0.6 ± 0.2 at 4 hours post-injection to 5.25 ± 1.1 at 72 hours postinjection.

Discussion

In the present study, a pretargeting system for CEA-expressing tumors was developed and characterized. Quadroma cells, producing bsMAb MN-14xDTIn-1 were prepared by fusion of two hybridoma cells: MAb MN-14 (anti-CEA) and MAb DTIn-1 (anti-DTPA(In)). The bsMAb was purified from cultured supernatants by a combination of protein A chromatography, cation-exchange chromatography, and by anti-CEA (WI-2) affinity column chromatography. At optimized conditions (10 μg bsMAb, 72-hour interval, and 6 ng bivalent peptide), LS174T tumors could be targeted efficiently with various bivalent peptides, labeled with different radionuclides.

The optimal injection ratio was 15:1 in these pretargeting studies with LS174T tumors for diagnostic applications, focused mainly on high T/B ratios (bsMAb/^{111}In-labeled bivalent peptide) with a 72-hour interval. For the treatment of tumors (RIT), the strategy should focus more on the maximum uptake of the radiolabel in the tumor and therefore, the injection ratio should be investigated further. These optimal injection ratios were in the same order of magnitude as the ratios found in previous pretargeting studies in renal cell carcinoma models using the IgG G250xDTIn-1 bsMAb (injection ratio 23:1; ref. 27). The maximum uptake of the ^{111}In-label in bsMAb MN-14xDTIn-1 pretargeted LS174T tumors was 17.7 ± 2.1% ID/g, which was comparable to the uptake reported by groups studying CEA-expressing tumors with chemically produced bispecific anti-CEA × anti-hapten F(ab′)$_2$-fragments (29.2 ± 5.1% ID/g; ref. 28; 19.8 ± 6.3% ID/g; ref. 24; 10.4 ± 1.6 % ID/g; ref. 29). These results were in accordance with the results described previously with respect to the pretargeting strategy of SK-RC-1 renal cell carcinoma model (27). Pretargeting of renal cell carcinoma using intact bsMAb IgG versus bsMAb F(ab′)$_2$ G250xDTIn-1 revealed that, at optimized conditions for each approach, the uptake of the radiolabeled peptide in the SK-RC-1 tumor was independent on the bsMAb form.

The uptake of the ^{111}In-labeled L-amino acid peptide in CEA-expressing tumors was considerably lower than that of the ^{111}In-label in various renal cell carcinomas pretargeted with bsMAb G250xDTIn-1 (23). This could be caused by the relatively high vascular volume of the renal cell carcinomas (20.8 ± 4.1 to 33.7 ± 5.9 μL blood/g) as compared with the vascular volume of LS174T tumor (11.8 ± 3.8 μL blood/g; ref. 23).

The uptake of the ^{111}In-label in the tumor at 24 hours postinjection obtained in different experiments (10 μg bsMAb, 72-hour interval, and 6 ng ^{111}In-diDTPA) varied considerably.

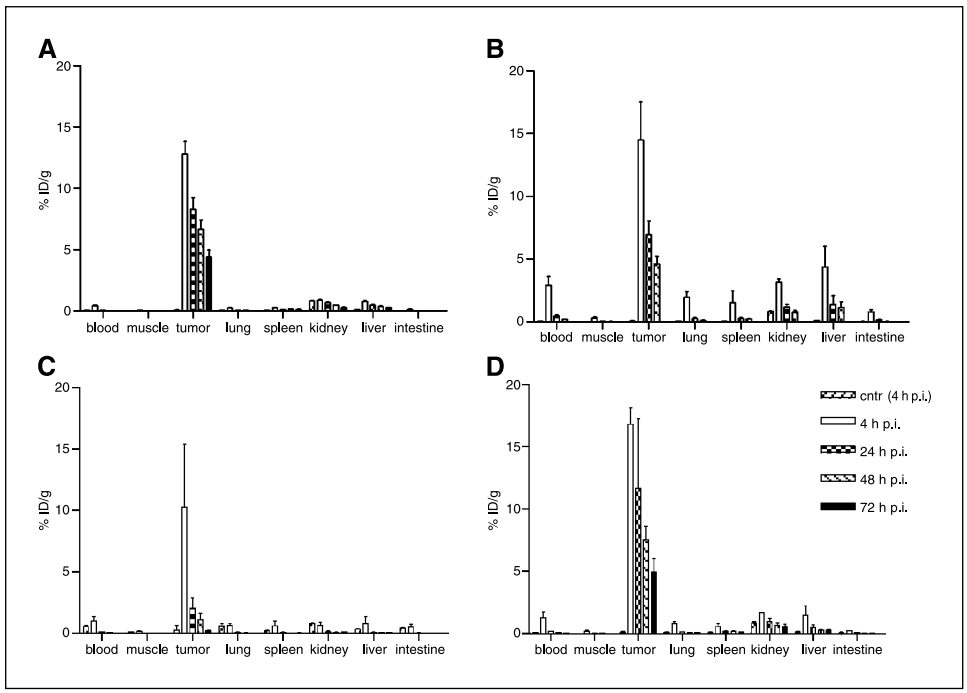

Fig. 5. Biodistribution results of bivalent peptides radiolabeled with different radiolabels 72 hours after pretargeting of LS174T tumors with bsMAb MN-14xDTIn-1. *A*, ^{111}In-L-amino acid peptide (average tumor size, 0.34 g); *B*, ^{99m}Tc-L-amino acid peptide (average tumor size, 0.18 g); *C*, ^{125}I-L-amino acid peptide (average tumor size, 0.19 g); and *D*, ^{125}I-D-amino acid peptide (average tumor size, 0.14 g). The uptake was expressed as % ID/g.

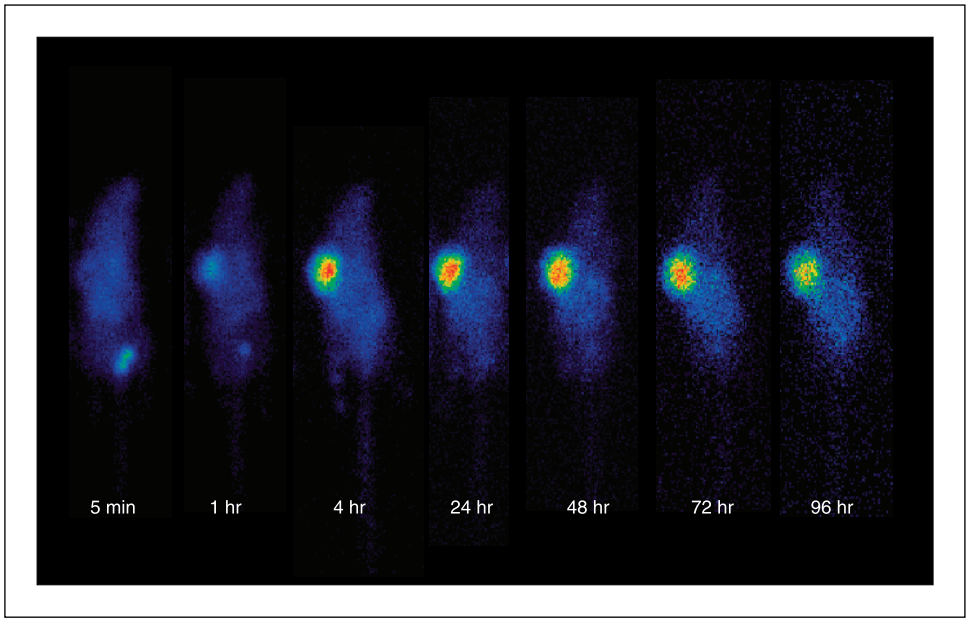

Fig. 6. Scintigraphic images of LS174T tumor – bearing mice pretargeted with 10 μg of bsMAb MN-14xDTIn-1, and 72 hours later, mice were injected with 3.7 MBq (6 ng) ^{111}In-labeled L-amino acid peptide. Scintigraphic images were recorded at 5 minutes, 1, 4, and 24 hours (300,000 counts), 48 hours (200,000 counts), 72 hours (100,000 counts), and 96 hours (75,000 counts) after the injection of the radiolabel.

These differences are most likely caused by the experiment-to-experiment variation in tumor size in the mice used in the various experiments. LS174T tumors are rapidly growing tumors which develop a necrotic center when they grow beyond the size of ~200 mm^3.

The biodistribution of various radionuclides was similar at 4 hours after administration of the radiolabeled diDTPA peptide (Fig. 5). Each of the four radiolabeled peptides used in these studies cleared from the tumor. CEA-bsMAb complexes on LS174T cells are internalized only to a very limited extent (30). Still, the residualizing radionuclide In-111 labeled to L-amino acid peptide as well as the residualizing ^{125}I-D-amino acid peptide were significantly better retained in the tumor as compared with L-amino acid peptide ^{125}I label. Previous experiments showed that in the SK-RC-52 renal cell carcinoma model, the biodistribution of D-amino acid peptides was very similar regardless of whether the radionuclide was I-125 or In-111 (15). When peptides are bound by the bsMAb on the surface of the cell, they might be degraded by peptidases present in necrotic tumors. The metabolite ^{125}I-Tyr formed after degradation of the ^{125}I-L-amino acid peptide is cleared from the tumor, whereas the metabolite ^{111}In-DTPA-Lys from the ^{111}In-labeled L-amino acid peptide could still be bound monovalently to bsMAb at the cell surface. The ^{125}I label could be better retained in the tumor when labeled to a D-amino acid peptide because the D-amino acid peptide bonds are more resistant to degradation by peptidases and therefore could remain intact at the tumor cell surface.

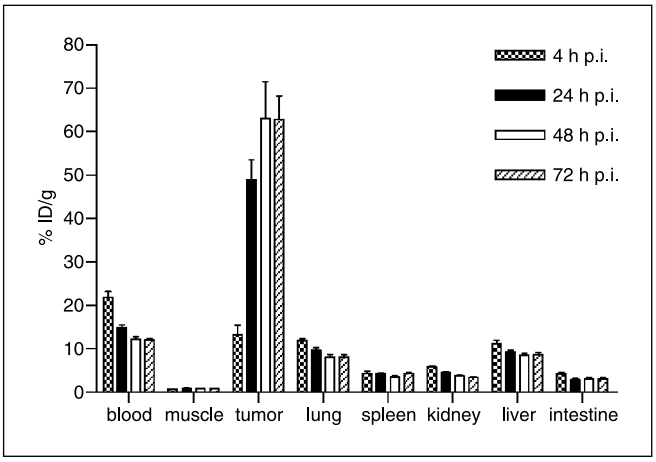

Fig. 7. Biodistribution results of directly labeled MAb MN-14. LS174T tumor – bearing mice were injected i.v. with 10 μg of ^{111}In-MN-14, and in time, mice were killed and the biodistribution of the radiolabel was determined. The uptake was expressed as % ID/g (average tumor size, 0.12 g).

The uptake of the ^{111}In-label in LS174T tumors, when done with directly labeled MN-14 was 5-fold higher than when done with the pretargeting strategy using the ^{111}In-labeled peptide. However, the T/B ratio with the pretargeting approach was, at 4 hours postinjection of the radiolabel, already significantly higher than with directly labeled MN-14 (34.4 ± 16.3 and 0.6 ± 0.2, respectively). This difference in T/B ratios increased at later time points, 445 ± 91 and 5.3 ± 1.1, respectively, at 72 hours postinjection.

In summary, an effective pretargeting strategy for CEA-expressing tumors was developed using a newly developed bsMAb MN-14xDTIn-1 and a radiolabeled bivalent peptide. Targeting of pretargeted colorectal cancer with the newly developed peptide consisting of D-amino acids enhanced the retention of iodinated peptides as compared with the use of iodinated L-amino acid peptides. The maximum uptake of directly labeled MN-14 in LS174T tumors was 5-fold higher as compared with the targeting observed with the pretargeting approach. However, compared with ^{111}In-labeled MN-14, radiolabeled diDTPA with the pretargeting strategy showed a major improvement of the T/B ratios, caused by the fast clearance of the radiolabeled peptide from the circulation.

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Pretargeting of Carcinoembryonic Antigen–Expressing Tumors with a Biologically Produced Bispecific Anticarcinoembryonic Antigen × Anti-Indium–Labeled Diethylenetriaminepentaacetic Acid Antibody

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