$CaNa_2EDTA$ for Improvement of Radioimmunodetection and Radioimmunotherapy with ^{111}In and ^{90}Y-DTPA-Anti-CEA MAbs in Nude Mice Bearing Human Colorectal Cancer

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^{111}In and ^{90}Y, dissociated from ^{111}In-labeled-monoclonal antibody (MAb) and ^{90}Y-labeled MAb, may cause deterioration of the image quality in radioimmunodetection (RID) and undesirable irradiation of nontargeted tissue in radioimmunotherapy (RIT), respectively. The aim of this study was to investigate any improvement in RID and RIT with ^{111}In-MAb and ^{90}Y-MAb by pre- and postadministration of calcium disodium ethylenetriaminetetraacetic acid ($CaNa_2EDTA$). **Methods:** Murine MAb F33-104 against carcinoembryonic antigen (CEA) was labeled with ^{111}In or ^{90}Y by the diethylenetriamine pentaacetic (DTPA)-anhydride method. The influence of $CaNa_2EDTA$ on loss of radioactivity from ^{111}In-MAb or ^{90}Y-MAb in serum was investigated in vitro. The effects of $CaNa_2EDTA$, administered before and after ^{111}In-MAb or ^{90}Y-MAb, on the biodistribution of radioactive isotopes in nude mice bearing human colon adenocarcinoma LS 180 tumor expressing CEA, or human pulmonary carcinoma PC 9 tumor expressing no CEA, were then examined. As a control, 0.9% NaCl was used in both the in vitro and in vivo studies. **Results:** $CaNa_2EDTA$ did not cause any decrease in levels of radioactivity of radiolabeled MAbs. Pre- and post-treatment with $CaNa_2EDTA$ reduced radioctivity in both specific and nonspecific tumors at 72 h after ^{111}In-MAb injection resulting in an increase of the specific tumor-to-nonspecific tumor radioactivity ratio. The levels of hepatic and renal radioactivity were also subsequently decreased by $CaNa_2EDTA$. On the other hand, $CaNa_2EDTA$ pre- and post-treatment reduced levels of bony, hepatic, and renal radioactivity at 24, 72, and 72 h, respectively, after ^{90}Y-MAb injection, although it had no effect on tumor radioactivity. **Conclusion:** Pre- and post-treatment with $CaNa_2EDTA$ would be of great use in humans who undergo RID or RIT with ^{111}In-MAb and ^{90}Y-MAb accompanied by disassociation of the labeled radionuclides.

Key Words: ^{111}In; ^{90}Y; calcium disodium ethylenediaminetetraacetic acid; radioimmunodetection; radioimmunotherapy

J Nucl Med 2000; 41:337–344

Received Jan. 19, 1999; revision accepted Jun. 21, 1999.

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Radioimmunodetection (RID) involves finding biologic markers in malignant tumor cells and may serve as a confirmatory test for growing neoplasms. It can also serve to qualify a patient for radioimmunotherapy (RIT) using the same antibody but a different isotope (*1*). ^{111}In is a γ emitter at 171 and 245 keV, with a half-life of 67.9 h. ^{90}Y is a β emitter at a maximum energy of 2.3 MeV, with a half-life of 64.0 h. ^{90}Y is known to have certain radiolabeling characteristics in common with ^{111}In. Therefore, a combination of ^{111}In and ^{90}Y with monoclonal antibodies (MAbs) may play a significant role in RID and RIT of various malignant diseases.These radioactive metals are necessary as an appropriate chelator to label MAb. Diethylenetriamine pentaacetic dianhydride (DTPA-anhydride) has been used as a chelator for covalent attachment to an MAb and for long-term radionuclide labeling (*2–4*). However, release of ^{111}In from ^{111}In-DTPA-MAb in blood may result in transport by transferrin to the liver and other organs of the reticuloendothelial system. The liver uptake poses a significant problem that drastically limits the usefulness of ^{111}In in clinical trials, because many carcinomas metastasize to the liver (*1,5*). Bone marrow activity may also interfere with the detection of bone metastases (*6*). Renal radioactivity of ^{111}In-MAb may cause artifacts in the upper abdomen and make routine clinical use more problematic. On the other hand, ^{90}Y, leaked from ^{90}Y-DTPA-MAb in blood, can accumulate in bone, because ^{90}Y is itself a bone-seeking agent (*1,7*). The bone uptake may cause myelosuppression. To improve RID and RIT with ^{111}In- and ^{90}Y-DTPA-MAbs, several strategies have been investigated, including the use of avidin-biotin labeling techniques (either biotinylated antibodies and ^{111}In-labeled avidin or streptoavidin, or streptavidin-conjugated MAb followed by injection of radiolabeled biotin) (*8–11*), to develop metal chelates that remain intact during the catabolism of conjugated MAb, and thus ensure the excretion of the labeled species (*12–16*), or to combine with autologous

bone marrow transplantation (*17*). A few chelates for labeling may have immunogenic properties (*5,16*). Calcium disodium ethylenetriaminetetraacetic acid ($CaNa_2EDTA$), which has been clinically used for treatment of heavy metal poisoning, may eliminate several metallic radionuclides from the body effectively. The pharmacologic effects of $CaNa_2EDTA$ result from the formation in the body of chelates with divalent and trivalent metals. Accessible metal ions (both exogenous and endogenous) with a higher affinity for $CaNa_2EDTA$ than calcium will be chelated, mobilized, and excreted (*18*). $CaNa_2EDTA$ has been reported to facilitate elimination of ^{111}In from mice bearing human tumor cells prelabeled with ^{111}In-oxine (*19*). It also has been shown that the administration of $CaNa_2EDTA$ substantially increased the tumor-to-muscle radioactivity ratio in nude mice injected with ^{111}In-labeled MAb (*20*). $CaNa_2EDTA$ has been shown in human subjects and mice to accelerate ^{90}Y excretion by chelating the released ^{90}Y without compromising the tumor uptake of ^{90}Y-labeled MAb and to allow an increase in the administered dosage of ^{90}Y-labeled MAb, as a result of the expected reduction in myelotoxicity (*21,22*). In this study, the possibility of improvement of RID and RIT with ^{111}In- and ^{90}Y-DTPA-MAb with $CaNa_2EDTA$ pre- and post-treatment was investigated, and the efficacy is discussed.

MATERIALS AND METHODS

Cell Lines and Animal Models

A human colon adenocarcinoma LS 180 cell line expressing carcinoembryonic antigen (CEA) was obtained from the American Type Culture Collection (Rockville, MD) and maintained in Eagle's minimum essential medium with nonessential amino acids in Earle's Balanced Salt Solutions supplemented with 10% fetal bovine serum (FBS). A human pulmonary squamous cell carcinoma PC 9 cell line expressing no CEA was cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% FBS. Female nude mice with a BALB/c/nu/nu background were obtained from the Institute of Experimental Animal Research, Gunma University School of Medicine (Gunma, Japan) at 4 wk of age and inoculated subcutaneously in the right rear flank with tumor cells (1×10^7 cells/mouse). Mice were allowed free access to commercial chow and distilled, deionized water during the experiment. Ambient temperature during the study was maintained at 21–23°C, and the mice were exposed to a 12-h light, 12-h dark cycle. Mean tumor weight was <0.50 g. Animal studies were performed in compliance with relevant laws relating to the conduct of animal experiments issued by the Gunma University Ethical Committee.

Radiolabeling of MAb

Murine MAb F33-104 (IgG1) was generated by a conventional hybridoma method with highly purified CEA from liver metastases of colorectal cancer as an immunizing antigen and reacted against B3 of protein epitopes on the CEA (*23,24*). MAb F33-104 (6.67×10^{-5} mmol) in 2 mL 0.1 mol/L $NaHCO_3$ was reacted with DTPA-anhydride (DOJIN, Kumamoto, Japan) (23.3×10^{-5} mmol) in dimethyl sulfoxide at 4°C for 12 h. These reaction conditions gave 1.8–2.1 DTPA groups per MAb molecule, determined by ^{111}In labeling of the crude mixture. The conjugation of DTPA with MAb in a 3.5:1 molar ratio caused the maximum loss of 20% in immunoreactivity of Mab, although the condition gave the MAb the optimum labeling efficiency in the combined labeling of ^{111}In and ^{90}Y. The DTPA-conjugated MAb was purified from unconjugated chelate molecules by Sephadex G25 gel filtration (Pharmacia, Uppsala, Sweden) using 0.1 mol/L $NaHCO_3$ as an eluant and was dialyzed in 50 mmol/L phosphate-buffered saline (PBS), pH 7.5, at 4°C for 24 h. For ^{111}In labeling, 500 μg DTPA-conjugated MAb in 0.1 mol/L sodium acetate buffer, pH 5.8, was mixed with ^{111}In-chloride (74 MBq/mL; Nihon Medi-Physics Co. Ltd., Nishinomiya, Japan) and allowed to react at room temperature for 60 min. After the addition of disodium salt EDTA (Na_2EDTA; DOJIN) to a final concentration of 5 mmol/L to chelate unbound ^{111}In, the ^{111}In-MAb was purified by Sephadex G25 gel filtration using 0.1 mol/L sodium acetate buffer, pH 5.8. For ^{90}Y labeling, 500 μg DTPA-conjugated MAb in 0.1 ml/L sodium acetate buffer, pH 5.8, was mixed with ^{90}Y-acetate (925 MBq/mL), which was obtained by incubation of an equal volume of 1 mol/L sodium acetate buffer, pH 5.8, and ^{90}Y-chloride (Nordion; Kanata, Ontario, Canada) for 30 min at 37°C and allowed to react at room temperature for 60 min. After the addition of EDTA, the ^{90}Y-MAb was purified by Sephadex G25 gel filtration. The purified ^{111}In-MAb and ^{90}Y-MAb contained <2% free radioactive ^{111}In and ^{90}Y, respectively, and the specific levels of radioactivity were calculated as approximately 74 MBq/mg. For a control, MAb F33-104 was labeled with ^{125}I by a chloramine-T method (specific activity, 440 MBq/mg) (*24,25*).

In Vitro Influence of Serum on ^{111}In-MAb or ^{90}Y-MAb

^{111}In-MAb or ^{90}Y-MAb was incubated with human normal serum or 50 mmol/L PBS, pH 7.5, as a control for 0, 3, 24, 72, 120, and 168 h at 37°C in an atmosphere of 5% carbon dioxide in air (10.4 μg MAb/mL). The influence of serum on the radioactivity of radiolabeled MAbs was estimated by cellulose diacetate electrophoresis as described (*4,24,26*). Cellulose diacetate (Separax-S; Fuji Photo Film Co. Ltd., Tokyo, Japan) was cut into 110 × 10 mm strips, soaked in 0.06 mol/L barbital buffer solution, pH 8.6. The strips then were laid between the anode and cathode in a flat bed electrophoresis tank. Each 1-μL sample was applied to the midpoints of the strips using a sample applicator for high-performance liquid chromatography (HPLC). ^{111}In-acetate, ^{111}In-DTPA, ^{90}Y-acetate, and ^{90}Y-DTPA incubated with serum (to approximately 740 kBq/mL) were also subjected to electrophoresis. The radiolabeled chelates were obtained by direct mixtures of chelators and radionuclides for 60 min at room temperature. After electrophoresis under constant current of 0.8 mA/strip for 30 min at room temperature, the strips were dried, cut into smaller 5 × 10 mm strips, and put into plastic tubes. The radioactivity of fractions was counted with a NaI well scintillation γ counter (Aloka, Tokyo, Japan). A 10% energy window was centered over the 245 keV of ^{111}In. For the ^{90}Y count, a window was set at 100 keV to infinity, and the counting efficiency of the NaI well scintillation γ counter was approximately 12%. After subtraction of levels of radioactivity corresponding to free radionuclides and chelated radionuclides, the radioactivity remaining at the MAb was calculated as a percentage of the radioactivity of the whole strip at each incubation time. The study was repeated 5 times in duplicate.

In Vitro Influence of $CaNa_2EDTA$ on ^{111}In-MAb or ^{90}Y-MAb

Athymic murine serum was mixed with $CaNa_2EDTA$ (Bleian Inj.; Nisshin Pharmacy Co. Ltd., Yamagata, Japan) to a final concentration of 1.6 mg/mL, corresponding to twice the level of the

initial serum concentration in a biodistribution study described below, or to 0.9% NaCl as a control. ^{111}In-MAb or ^{90}Y-MAb was then incubated with the mixture at 37°C for 0, 3, 24, 72, 120, and 168 h in an atmosphere of 5% carbon dioxide in air (10.4 μg MAb/mL serum). The influence of chelator on the radioactivity of radiolabeled MAbs was estimated by cellulose diacetate electrophoresis using the same method as that described above. ^{111}In-EDTA and ^{90}Y-EDTA incubated with serum (to ~740 kBq/mL) were also subjected to electrophoresis as additional references.

In Vitro Influence of $CaNa_2EDTA$ on ^{111}In-MAb or ^{90}Y-MAb Bound to Tumor Cells

LS 180 cells were grown to approximately 80% confluence in 4 dishes (150 × 150 mm) and were preincubated with ^{111}In-chloride (740 kBq) in 20 mL fresh medium supplemented with 10% FBS for 60 min at 37°C in an atmosphere of 5% carbon dioxide in air, to avoid the influence of incorporation of radionuclides into tumor cells (prelabeling). After triple washing with 20 mL fresh medium, 1 μL of the mixture of ^{111}In-MAb and serum (10.4 μg MAb/mL) incubated for 72 or 168 h at 37°C was added into the dishes and incubated at 37 °C for 60 min. The cells were then incubated with 32 mg $CaNa_2EDTA$ (1.6 mg/mL) or 0.9% NaCl as a control at 37°C for 60 min in air after triple washing with 20 mL fresh medium. After collection of 1 × 10^7 cells in 200 μL PBS (136.8 mmol/L NaCl, 2.7 mmol/L KCl, 8.1 mmol/L Na_2HPO_4, and 1.5 mmol/L KH_2PO_4) in microtubes, the cells were separated by centrifugation at 10,000*g* for 5 min and were counted in a NaI well scintillation γ counter. Results were expressed as the mean ± SD of [counts/min of cells with $CaNa_2EDTA$]/[counts/min of cells with 0.9% NaCl] × 100) (%) from a 5-time interassay in duplicate. The same procedure, except for prelabeling, was performed for ^{90}Y-MAb. Cell viability was ascertained at >95% by trypan blue staining.

Effect of $CaNa_2EDTA$ on Biodistribution of LS 180 Tumor- or PC 9 Tumor-Bearing Nude Mice with ^{111}In-MAb or ^{90}Y-MAb

$CaNa_2EDTA$ (37.5 mg/kg) was slowly administered intraperitoneally before and after intravenous injection of ^{111}In-MAb or ^{90}Y-MAb (500 μg MAb/kg of mouse body weight; specific activity, 74 MBq/mg) to nude mice bearing LS 180 tumor or PC 9 tumor. As a control, 0.9% NaCl was used in place of the chelator. Mice were killed under general anesthesia with diethylether (Wako, Osaka, Japan) at 3, 24, 72, 120, and 168 h after intravenous injection of ^{111}In-MAb or ^{90}Y-MAb. The protocol is shown in Figure 1. Circulating blood was replaced by 0.9% NaCl. The right atrium was cut and opened, and, after sampling blood by a heart puncture, 0.9% NaCl was gently perfused into the left ventricle with a small syringe. The tumor and organs were removed and cut into pieces weighing <200 mg, and the volume of the blood sample was <100 μL. The levels of radioactivity of each piece of tumor, of organs, and of blood were counted in a NaI well scintillation γ counter. Under this condition, a sufficient linearity between ^{90}Y radioactivity and counting rate was obtained. Results were expressed as percentage injected dose/gram tissue (%ID/g) normalized to a 20-g mouse for each tissue radioactivity. Specific tumor-to-normal tissue radioactivity ratio was obtained from ([%ID/g LS 180 tumor]/[%ID/g tissue]). Specific tumor (LS 180 tumor)-to-nonspecific tumor (PC 9 tumor) radioactivity was calculated as ([mean %ID/g LS 180 tumor]/[mean %ID/g PC 9 tumor]). Serum calcium levels from blood of mice with $CaNa_2EDTA$ or 0.9% NaCl were monitored at 168 h after injection by the orthocresol phthalein complexone method. Circulating CEA concentration in the blood of LS 180 tumor- or PC 9 tumor-bearing mice at various points during the study was determined by CEA-immunoradiometric assay kit Daiichi II (Daiichi Radioisotope Laboratory, Tokyo, Japan).

Data Analysis

Statistical comparisons were made by the Student *t* test. Differences were considered statistically significant when $P < 0.05$.

RESULTS

In Vitro Studies

Loss of radioactivity from ^{111}In-MAb or ^{90}Y-MAb in human serum was approximately 10% and 33%, respectively, for up to 168 h, whereas loss of radioactivity from ^{111}In-MAb and ^{90}Y-MAb in PBS was approximately 3% and 5%, respectively (Fig. 2A). No influence of $CaNa_2EDTA$ on loss of radioactivity from ^{111}In-MAb or ^{90}Y-MAb was shown (Fig. 2B). There was no difference in loss of radioactivity from radiolabeled MAbs between human serum and murine serum. No effect of $CaNa_2EDTA$ on loss of radioactivity

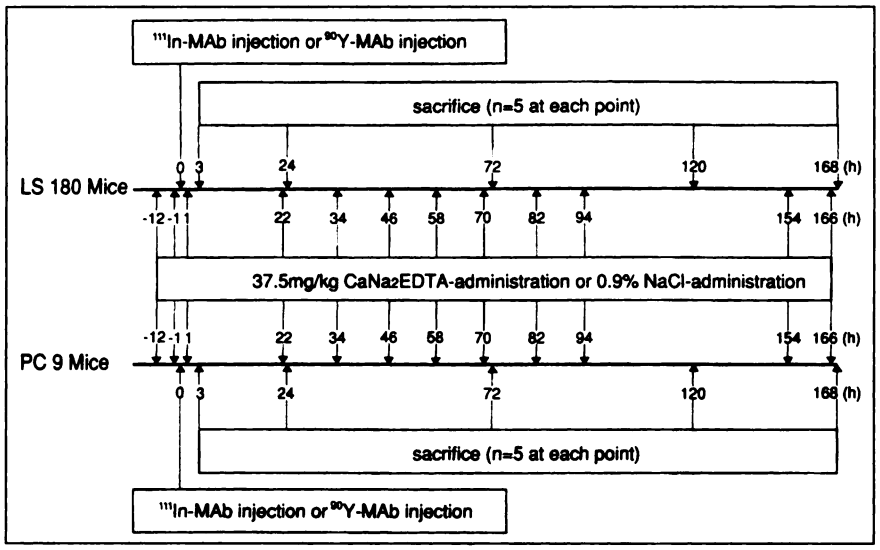

FIGURE 1. Protocol of in vivo studies.

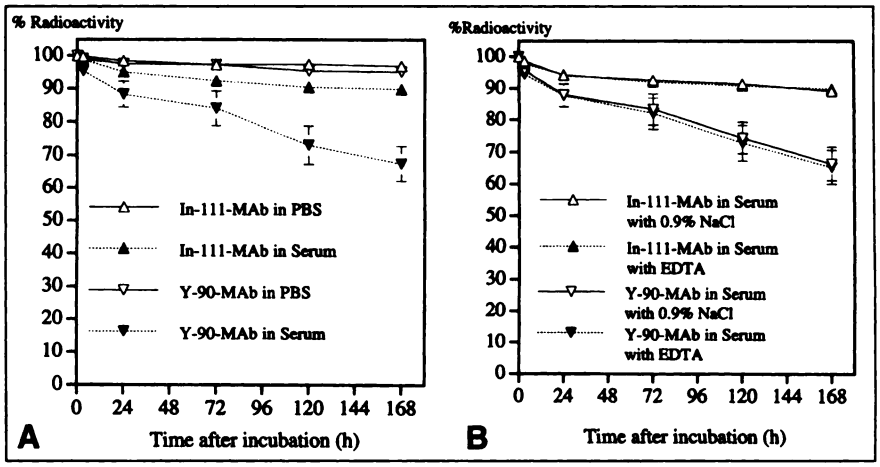

FIGURE 2. (A) Loss of radioactivities from ^{111}In-MAb and ^{90}Y-MAb in serum. (B) Influence of $CaNa_2EDTA$ on loss of radioactivities from ^{111}In-MAb and ^{90}Y-MAb in serum.

from ^{111}In-MAb or ^{90}Y-MAb binding to LS 180 cells was shown (99.4% ± 0.57% for 72-h mixture versus 99.2% ± 0.39% for 168-h mixture; 98.4% ± 1.48% versus 98.3% ± 1.42%, respectively).

In Vivo Studies

$CaNa_2EDTA$ significantly decreased the %ID/g of tumor in LS 180 tumor-bearing mice from 72 h after injection of ^{111}In-MAb compared with that of the control (Fig. 3). In addition, the hepatic and renal levels of radioactivity in $CaNa_2EDTA$-treated mice bearing LS 180 tumor were also significantly reduced compared with those of controls (Fig. 3). With respect to other tissues or blood, $CaNa_2EDTA$ had no influence on the radioactive levels (%ID/g). The tumor-to-blood ratio in mice bearing LS 180 tumor was significantly decreased from 72 h after injection of ^{111}In-MAb by $CaNa_2EDTA$ compared with that of the control (Table 1), but the tumor-to-liver or tumor-to-kidney radioactivity ratios were not affected (Fig. 4). On the other hand, in mice bearing

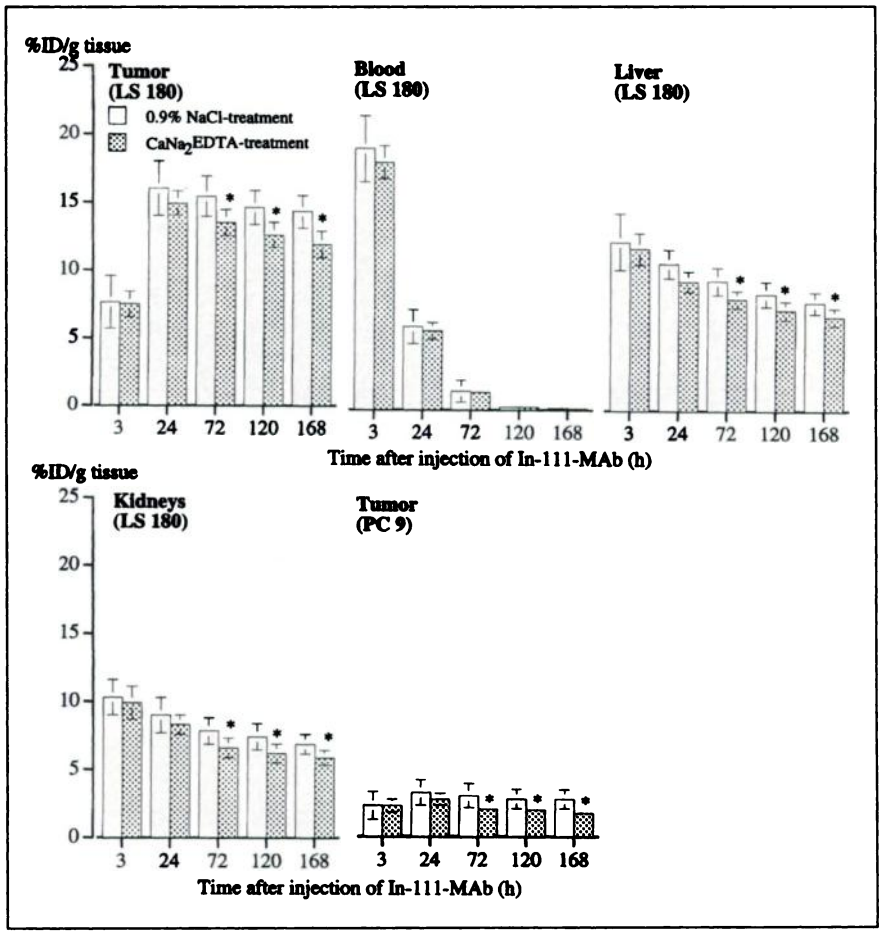

FIGURE 3. Effect of pre- and postadministered $CaNa_2EDTA$ on biodistribution of radioactivity in tumor-bearing mice injected with ^{111}In-MAb. $^*P < 0.05$ for $CaNa_2EDTA$-treated mice and controls.

TABLE 1
Effect of Pre- and Postadministered $CaNa_2EDTA$ on Tumor-to-Blood Radioactivity Ratio in LS 180 Tumor-Bearing Mice Injected with ^{111}In-MAb or ^{90}Y-MAb

Time after injection (h)	^{111}In-MAb		^{90}Y-MAb	
	0.9% NaCl treatment	$CaNa_2EDTA$ treatment	0.9% NaCl treatment	$CaNa_2EDTA$ treatment
3	0.35 ± 0.07	0.36 ± 0.04	0.69 ± 0.10	0.73 ± 0.12
24	2.28 ± 0.38	2.24 ± 0.19	4.44 ± 0.23	4.60 ± 0.36
72	9.65 ± 0.93	8.49 ± 0.61*	36.2 ± 5.52	37.9 ± 4.05
120	58.2 ± 5.37	50.6 ± 4.90*	42.0 ± 6.15	42.8 ± 5.20
168	89.6 ± 6.75	79.3 ± 6.60*	53.2 ± 7.40	54.6 ± 7.13

*$P < 0.05$ between 0.9% NaCl-treated (control) mice and $CaNa_2EDTA$-treated mice.
Values are mean ± SD.

PC 9 tumor, $CaNa_2EDTA$ significantly reduced the %ID/g of tumor from 72 h after injection of ^{111}In-MAb (Fig. 3). The specific tumor-to-nonspecific tumor radioactivity ratio was definitely raised from 72 h after injection of ^{111}In-MAb by $CaNa_2EDTA$ treatment compared with that of the control (Fig. 5).

$CaNa_2EDTA$ significantly decreased the bony radioactivity of mice bearing LS 180 tumor from 24 h after injection of

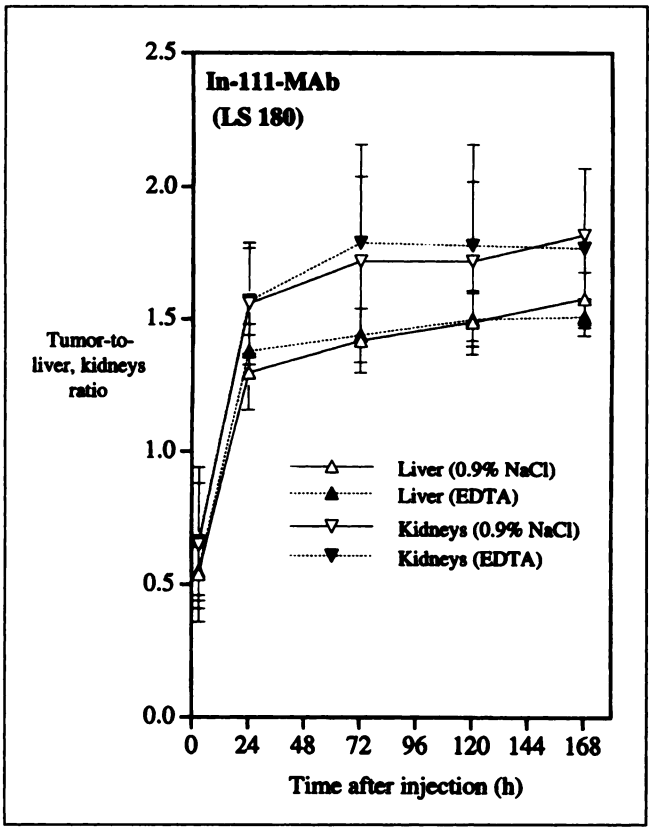

FIGURE 4. Effect of pre-and postadministered $CaNa_2EDTA$ on tumor-to-liver and -kidney radioactivity ratios in LS 180 tumor-bearing mice injected with ^{111}In-MAb.

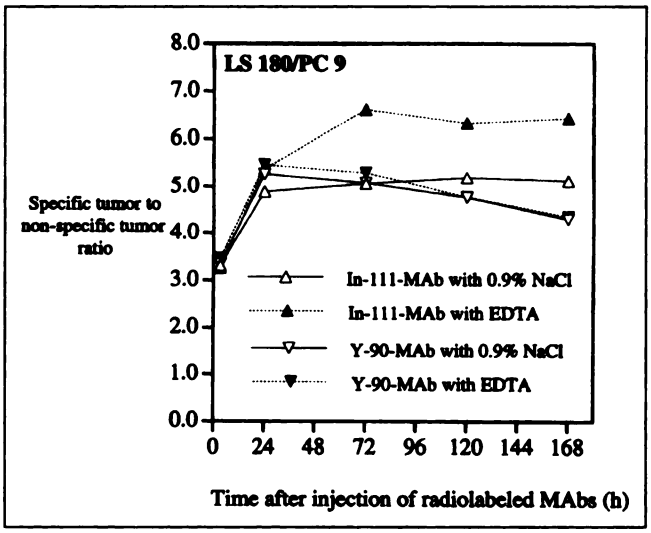

FIGURE 5. Effect of pre- and postadministered $CaNa_2EDTA$ on specific tumor-to-nonspecific tumor radioactivity ratio of ^{111}In-MAb or ^{90}Y-MAb.

^{90}Y-MAb compared with that of the control (Fig. 6). The hepatic and renal levels of radioactivity in LS 180 tumor-bearing mice were significantly reduced by $CaNa_2EDTA$ treatment from 72 h after injection of ^{90}Y-MAb, compared with that of the control mice (Fig. 6). $CaNa_2EDTA$ had no effect on %ID/g of tumor in LS 180 tumor-bearing mice (Fig. 6). With respect to other tissues or blood, $CaNa_2EDTA$ had no influence on the levels of radioactivity (%ID/g). $CaNa_2EDTA$ had no effect on tumor-to-blood radioactivity ratio in mice bearing LS 180 (Table 1). $CaNa_2EDTA$ significantly raised the tumor-to-bone, tumor-to-liver, and tumor-to-kidney radioactivity ratios in mice bearing LS 180 tumor from 24, 72, and 72 h, respectively, after injection of ^{90}Y-MAb, compared with those of controls (Fig. 7). On the other hand, in mice bearing PC 9 tumor, $CaNa_2EDTA$ had no effect on the %ID/g of tumor tissue. The specific tumor-to-nonspecific tumor radioactivity ratio of ^{90}Y-MAb was not influenced by $CaNa_2EDTA$ (Fig. 5).

No significant growth of the LS 180 or PC 9 tumor was found during these studies. In all mice injected with $CaNa_2EDTA$, no inflammation of the abdominal wall was seen macroscopically. No weight loss or other sign of toxicity such as shivering was observed, nor was any macroscopic change observed in mice kidneys. No significant difference in concentration of serum calcium was noted between the $CaNa_2EDTA$-treated mice and the normal mice (4.62 ± 0.25 mEq/L, n = 39; 4.65 ± 0.15 mEq/L, n = 43). No circulating CEA was detected during the study in the blood of mice bearing LS 180 or PC 9 tumors (<1 ng/mL).

DISCUSSION

$CaNa_2EDTA$ did not deplete the levels of radioactivity of radiolabeled MAbs in vitro. In the tumor-bearing mice, $CaNa_2EDTA$ pre- and post-treatment reduced the radioactivity in both specific and nonspecific tumors at 72 h after

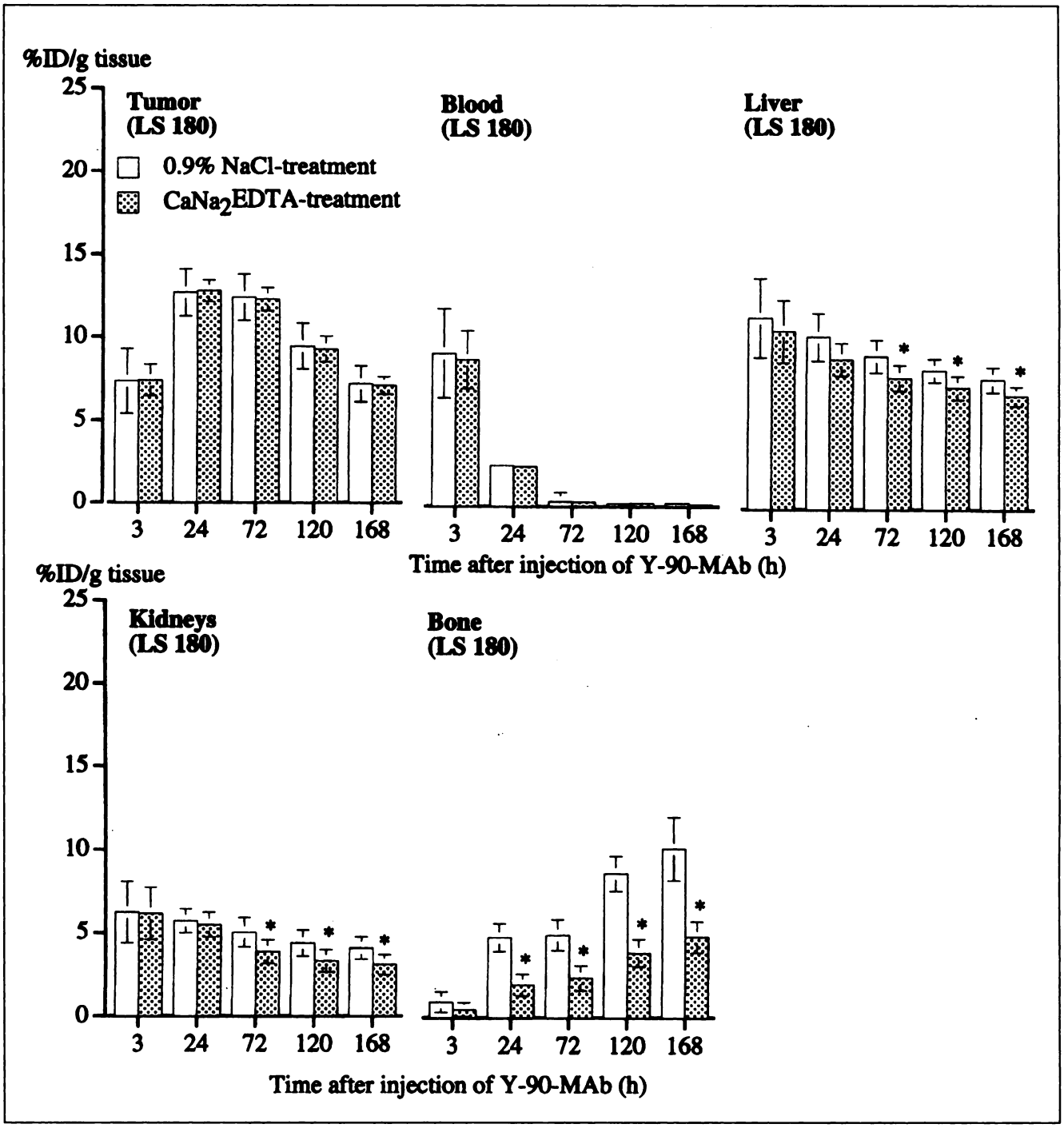

FIGURE 6. Effect of pre- and postadministered $CaNa_2EDTA$ on biodistribution of radioactivity in LS 180 tumor-bearing mice injected with ^{90}Y-MAb. *$P < 0.05$ for $CaNa_2EDTA$-treated mice and controls.

^{111}In-MAb-injection, and it raised the specific tumor-to-nonspecific tumor radioactivity ratio but lowered the specific tumor-to-blood radioactivity ratio. In the LS 180 tumor-bearing mice, pre- and postadministration of $CaNa_2EDTA$ decreased the hepatic and renal levels of radioactivity at 72 h after ^{111}In-MAb injection but did not affect the tumor-to-liver or -kidney radioactivity ratios. By contrast, in the tumor-bearing mice injected with ^{90}Y-MAb, pre-and post-treatment with $CaNa_2EDTA$ did not affect the radioactivity in either specific or nonspecific tumors, nor did it influence the specific tumor-to-nonspecific tumor radioactivity ratio or the tumor-to-blood radioactivity ratio. Pre- and postadministered $CaNa_2EDTA$ reduced the bony, hepatic, and renal levels of radioactivity in the LS 180 tumor-bearing mice at 24, 72, and 72 h after ^{90}Y-MAb injection and raised the specific tumor-to-bone, -liver, and -kidney radioactivity ratios. When not used in combination with preadministration, postadministration only of $CaNa_2EDTA$ had no effect

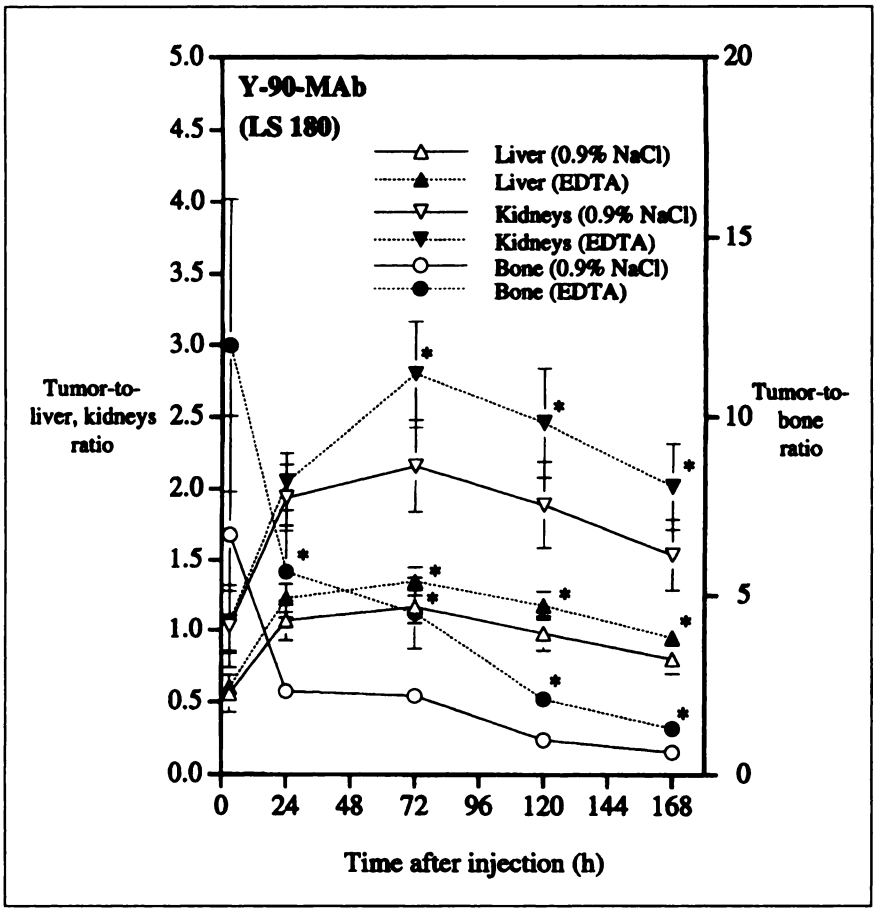

FIGURE 7. Effect of pre- and postadministered $CaNa_2EDTA$ on tumor-to-liver, -kidney, and -bone radioactivity ratios in LS 180 tumor-bearing mice injected with ^{90}Y-MAb. $^*P < 0.05$ for $CaNa_2EDTA$-treated mice and controls.

on raising the specific tumor-to-nonspecific tumor radioactivity ratio in ^{111}In-MAb, and the power of reduction in bony, hepatic, and renal levels of radioactivity of ^{90}Y-MAb was weakened (data not shown). The introduction of pretreatment with $CaNa_2EDTA$ showed an early effect on bony radioactivity for ^{90}Y-MAb. In tumor-bearing mice with ^{111}In-chloride and normal mice with ^{90}Y-acetate, pre- and postadministration of $CaNa_2EDTA$ was shown to more powerfully control the levels of radioactivity in the tumor and bone, liver, and kidneys, respectively, than postadministration only (unpublished data, Watanabe N, December 1998). Pretreatment with $CaNa_2EDTA$ also appears to be viable for the immediate removal of extracellular free radionuclides.

Soluble ^{111}In may localize in human malignant tumors without preference for cell type (*27,28*). The site of ^{111}In accumulation in tumor cells was the cytoplasmic lysosomes (*29*). Tumors include extracellular components such as connective tissue and vessels, in addition to cells, and various pathologic changes such as necrosis and inflammation. In tumors xenografted in nude mice, the actual distribution of ^{111}In was predominant in connective tissue (especially inflammatory tissue) rather than in viable tumor cells themselves (*30*). The ^{111}In was bound to acid mucopolysaccharides or the sulfated carbohydrate chain of sulfated glycoprotein produced as intercellular substances in inflammatory tissue (*30*). ^{111}In activity was rarely seen in necrotic tumor tissue. Therefore, it was reasonable to assume that the free ^{111}In disassociated from ^{111}In-MAb by catabolism or instability of the labeling (or both) or internalization was retained in the tumor, partly in the tumor cells and partly in the extracellular space. Furthermore, $CaNa_2EDTA$ treatment chelated the free, extracellular ^{111}In (and the $CaNa_2EDTA$ might inhibit the incorporation of ^{111}In into the tumor cells), resulting in reduced radioactivity in the tumor. The finding that free radioactivity from ^{111}In-labeled MAb may be retained in tumors was reported in the early stage of an investigation into RID (*31–33*). The free radionuclide dissociated from ^{90}Y-MAb has a strong potential for redistribution into bone. In a previous study we showed that $CaNa_2EDTA$ could not remove the ^{90}Y binding to the bone (unpublished data, Watanabe N, December 1998). Therefore, this $CaNa_2EDTA$-mediated effect on bony radioactivity was the result of chelation of the ^{90}Y liberated from the radiolabeled MAb before localization in bone. The early distribution of radioactivity in the liver and kidneys appeared to be the result of radiolabeled MAb itself binding to cells, through the Fc receptor in most cases. Sequentially, free radionuclides are a result of the catabolism and inherent instability of radiolabeled MAbs. Finally, both radiolabeled MAbs and freed radionuclides are nonspecifically distributed in the

liver and kidneys. It is assumed that $CaNa_2EDTA$ scavenges the free, extracellular radionuclides in the liver and kidneys.

Thus, pre- and post-treatment with $CaNa_2EDTA$ has an effect on the improvement of specificity of tumor radioactivity in RID, in addition to reducing hepatic and renal levels of radioactivity (improvement of imaging quality for diagnosis). On the other hand, pre- and postadministered $CaNa_2EDTA$ may permit a sufficient increase of ^{90}Y-MAb dosage for cancer treatment in RIT, because of the increase in the tumor-to-bone, tumor-to-liver, and tumor-to-kidney radioactivity ratios.

CONCLUSION

This study strongly suggests that pre- and post-treatment with $CaNa_2EDTA$ would be of great use for humans in both RID and RIT with ^{111}In- and ^{90}Y-MAb when radionuclides are freed from the radiolabeled MAbs.

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