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Radioimmunotherapy Targeting of HER2/neu Oncoprotein on Ovarian Tumor Using Lead-212-DOTA-AE1

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The specificity, toxicity and efficacy of lead (^{212}Pb) radioimmunotherapy were evaluated in nude mice bearing the SK-OV-3 human ovarian tumor cell line expressing the HER2/*neu* proto-oncogene. **Methods:** The therapeutic agent used was the tumor-specific anti-HER2/neu monoclonal antibody AE1 conjugated to ^{212}Pb, ^{212}Bi being the daughter and thus the source of the alpha-particle and beta emissions. A bifunctional derivative of tetraazacyclododecanetetraacetic acid (p-SCN-Bz-DOTA) was used to couple ^{212}Pb to the anti-HER2/neu monoclonal antibody AE1. The chelating agent did not alter the binding affinity to its antigenic target or the pharmacokinetics and tissue distribution of the AE1 antibody. Toxicity and therapeutic efficacy of ^{212}Pb-AE1 were evaluated in nude mouse ascites or solid tumor models, wherein SK-OV-3 cells were administered i.p. or s.c., respectively. **Results:** The dose-limiting acute toxicity after i.v. administration of ^{212}Pb-AE1 was bone marrow suppression, which was observed at doses above 25 μCi. Therefore, doses of 10 and 20 μCi were used in efficacy trials. The i.p. administration of ^{212}Pb-AE1 3 days after i.p. tumor inoculation led to a significant (p_2 = 0.015) prolongation of tumor-free survival. In a second model, i.v. treatment with ^{212}Pb-AE1 3 days after s.c. tumor inoculation prevented subsequent tumor development in all animals treated with 10 or 20 μCi of ^{212}Pb-AE1 (p_2 = 0.002 compared to control groups). This efficacy in the adjuvant setting was antibody specific because treatments with equivalently labeled control antibody or unlabeled AE1 antibody or no treatment were less effective. The rate of growth of small (mean tumor volume, 15 mm^3) SK-OV-3 tumors was modestly inhibited. However, tumor growth was not inhibited in mice bearing larger (mean tumor volume, 146 mm^3) SK-OV-3 tumors by the administration of a single dose of 10 or 20 μCi of ^{212}Pb-AE1. **Conclusion:** Lead-212-AE1 as an intact radiolabeled monoclonal antibody may be of only modest value in the therapy of bulky solid tumors due to the short physical half-life of ^{212}Pb and time required to achieve a useful tumor-to-normal tissue ratio of radionuclide after administration. However, the radiolabeled monoclonal antibody may be useful in therapy of tumors in the adjuvant setting. Furthermore, ^{212}Pb may be of value in select situations, including treatment of leukemia, intercavitary therapy or strategies that target vascular endothelial cells of tumors.

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Monoclonal antibodies targeted to cell surface antigens have been used to treat patients with a variety of cancers. However, unmodified monoclonal antibodies have been relatively ineffective (*1*). One of the factors in this low therapeutic efficacy is that most of the monoclonal antibodies used are not effective cytocidal agents against human neoplastic cells. Furthermore, in most cases, the antibodies were not directed against a vital structure on the surface of malignant cells, such as a growth factor receptor involved in tumor cell proliferation and survival. In an attempt to circumvent these problems, researchers have augmented the cytotoxic action of monoclonal antibodies by arming them with toxins or radionuclides (*2–6*). Furthermore, cell surface antigenic targets, especially receptors for growth factors, have been defined for more effective monoclonal antibody action. In particular, the HER2/neu receptor, the product of the c-*erb*B-2 proto-oncogene, has been the target of monoclonal antibody therapeutic trials (*7–12*). The HER2/*neu* oncogene (*erb*B-2) encodes a M_r 185,000 transmembrane phosphoglycoprotein. The HER2/*neu* gene is overexpressed in 20%–30% of adenocarcinomas of the breast, ovary, lung and stomach and has been linked to poor prognosis (*13–15*). A series of anti-HER2/neu monoclonal antibodies has been generated that includes antibodies without biological activity, as well as those with agonist action in terms of tyrosine phosphorylation of HER2/neu (*7–12*). Preclinical efficacy in mice bearing HER2/neu-expressing human tumor xenografts has been demonstrated with certain antibodies directed toward this receptor.

Although unmodified murine antibodies to the HER2/neu oncoprotein inhibited tumor growth in certain cases, in general it was not sufficient to cure animals of established tumors. This limited efficacy led to alternative approaches that include the humanization of an anti-HER2/neu antibody (*12,16*), the development of a humanized bispecific $F(ab')_2$ fragment for

retargeting cytotoxic T cells (*17,18*), a disulfide stabilized anti-HER2/neu *Pseudomonas* immunotoxin (*11*) and a disulfide-stabilized anti-HER2/neu Fv-beta-lactamase fusion protein for the activation of a cephalosporin doxorubicin prodrug (*19*). In addition, anti-HER2/neu antibodies have been used as carriers of toxic agents, including radionuclides. De Santes et al. (*10*) demonstrated that animals treated with 400–700 μCi of ^{131}I-anti-HER2/neu monoclonal antibodies manifested a marked inhibition of the growth of large tumors. In general, ^{131}I-labeled monoclonal antibodies have been relatively ineffective due to limitations of this radionuclide as a therapeutic agent.

In light of the limitations of radioiodine, metallic radionuclides that can be linked to antibodies may provide a better choice. Antibodies may be armed with beta- or alpha-emitting radionuclides. Future development of radionuclide-conjugated monoclonal antibodies may focus on radionuclides that emit alpha particles, which may be the most effective agents at killing tumor targets without damaging distant normal tissues (*2–6*).

Our present studies focused on the radionuclide ^{212}Pb, which has a 10.6-hr half-life. Lead-212 is the parent of ^{212}Bi, which is the source of an alpha particle as well as beta and gamma emissions. In this study, we used ^{212}Pb-tetraazacyclododecanetetraacetic acid-AE1 (^{212}Pb-DOTA-AE1) directed toward SK-OV-3 tumors in our therapeutic trials. The goals of this study were: to determine specificity and pharmacokinetics, including biodistribution of the DOTA chelate AE1 anti-HER2/neu monoclonal antibody in our experimental tumor model; to define the toxicity of the ^{212}Pb-DOTA-AE1 monoclonal antibody conjugate and, thereby, establish the maximum tolerated dose; and to define the efficacy, specificity and toxicity of ^{212}Pb radioimmunotherapy of a human HER2/neu-expressing tumor SK-OV-3 in nude mice.

MATERIALS AND METHODS

Cell Lines and Monoclonal Antibodies

The SK-OV-3 cell line (ATCC HTB 77), derived from a human ovarian adenocarcinoma, was used. AE1 is an IgG2a murine monoclonal antibody directed against the extracellular domain of the HER2/neu receptor. The previously described anti-Tac, an IgG2a murine monoclonal antibody that binds to the human IL-2Ralpha subunit, but not to murine SK-OV-3 cells, was used as a negative control antibody (*20*).

Radioiodination of Monoclonal Antibodies

Radioiodinations of AE1 for bindability studies with ^{125}I were performed using a modification of the chloramine-T method; the resultant specific activity was 4.0–6.5 μCi/μg. Ninety-seven percent of the iodinated AE1 was precipitable with 20% trichloroacetic acid.

Conjugation of the Chelate to Monoclonal Antibodies

To permit labeling with ^{212}Pb or ^{88}Y, the monoclonal antibody AE1, as well as anti-Tac, which served as the control antibody for all studies, was first conjugated with 2-(p-SCN-Bz)-DOTA (*21,22*). For convenience, the chelate will be referred to as DOTA. Typically, 8–10 mg of antibody at a concentration of about 5 mg/ml were dialyzed against bicarbonate buffer, pH 8.6, for 6 hr. The antibody preparation was then conjugated to ^{14}C-(p-SCN-Bz)-DOTA as described (*21,22*). The average number of DOTA chelates per molecule of antibody was 1.2, determined as described previously (*22*). Each lot of chelated AE1 antibody was compared with the unmodified antibody and was shown to have an unaltered binding capacity using a competitive binding assay (Fig. 1). Briefly, 5 × 10^5 SK-OV-3 cells were incubated on ice with

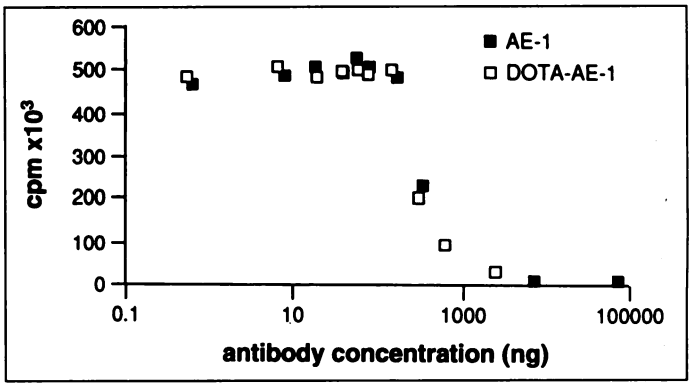

FIGURE 1. Assay of the effect of AE1 and DOTA-AE1 on the binding of ^{125}I-AE1 to SK-OV-3 cells. ■, unmodified competitor; □, DOTA-AE1 competitor.

increasing concentrations of unmodified (AE1) or chelated (DOTA-AE1) antibody (ranging from 0 to 75,000 ng/vial or from 0 to 2,688 ng/vial, respectively) in the presence of a constant nonsaturating amount of ^{125}I-AE1 antibody (6.25 ng/vial). After 3 hr of incubation, cells were centrifuged through an oil cushion, and cell-bound radioactivity was determined.

Procedure for Radiolabeling of Chelated Antibody

Carrier-free ^{88}Y was purified from metal contaminants by column extraction chromatography and incorporated into the chelated antibody as described (*23*).

Lead-212 was eluted from a ^{224}Ra/^{212}Pb generator with 1 ml of 2.0 *M* HCl. The solution was passed through a column (2 × 20 mm) of MP-50 resin, pre-equilibrated with 2.0 *M* HCl to remove any breakthrough of ^{224}Ra. The solution was then evaporated to dryness with the addition of 0.5 ml of concentrated HNO_3. The ^{212}Pb activity was dissolved in 0.1 *M* HNO_3, with the pH adjusted to ~4.0 with 3 *M* NH_4OAc. Chelated antibody was added to the ^{212}Pb solution and allowed to react at 35°C for 45 min. Five microliters of 0.1 m EDTA were added to scavenge any free radionuclide. Radiolabeled antibody was purified by high-performance liquid chromatography using a size-exclusion column and 4-morpholinepropanesulfonic acid/Cl buffer as the mobile phase (1 ml/min flow rate). Radiolabeling yields were 65%–75%, and more than 99% of the ^{212}Pb was protein-bound before administration.

Animals

Female 6–8-wk-old athymic-NCR-nude mice were kept in microisolation units on sterilized water and food.

Pharmacokinetics and Tissue Distribution of Radiolabeled AE1

To define the rate of entry of AE1 antibody into a HER2/neu-expressing tumor, mice bearing established s.c. injected SK-OV-3 tumors on their shoulder were injected i.v. with the 2-μCi dose of ^{88}Y-labeled monoclonal antibody. Seventeen mice were injected with the specific anti-HER2 antibody (^{88}Y-DOTA-AE1), and two mice were injected with an irrelevant (^{88}Y-DOTA-anti-Tac) antibody. Three of the mice injected with specific antibody were killed at 2, 6, 10 and 24 hr, and two mice were killed at 48 hr following infusion; their organs were removed for weighing and radioactivity determination using a gamma ray counter.

Toxicity of Lead-212

Lead-212 was used as the radionuclide in the immunotherapeutic studies. The emissions of ^{212}Pb, the decay product daughters and the details of the decay events as they relate to radioimmunotherapy have been described in the literature and are outlined in Figure 2 (*24*).

To establish the toxicity and maximal tolerated dose of the ^{212}Pb-DOTA-AE1, healthy, tumor-free mice were injected with

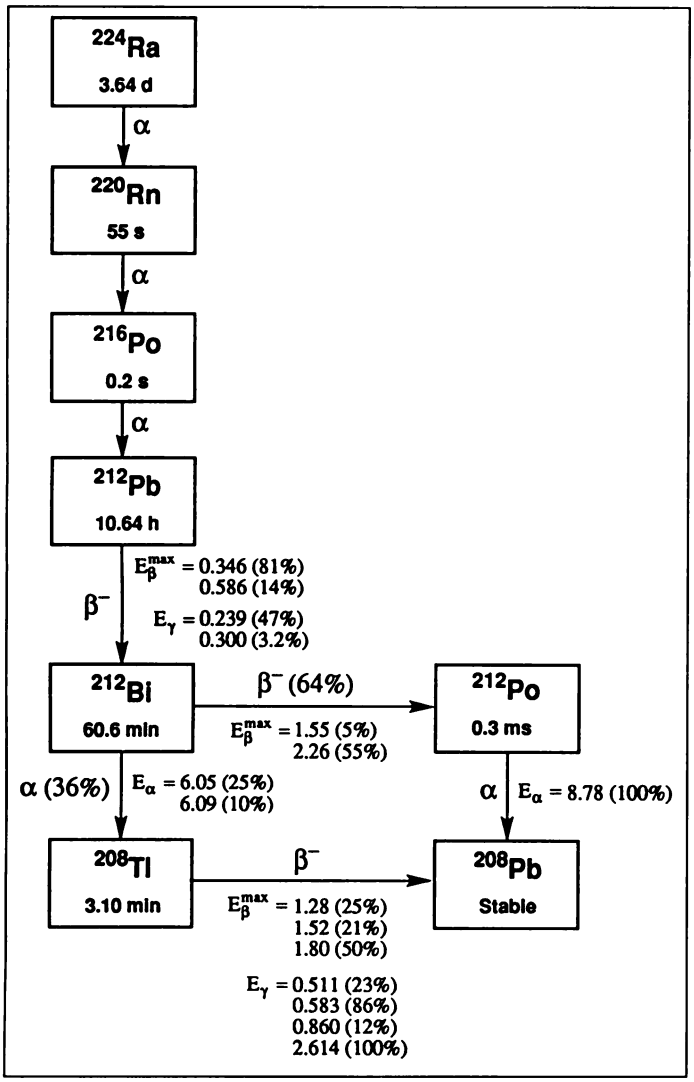

FIGURE 2. Decay series of ^{224}Ra for generating therapeutic doses of ^{212}Pb and ^{212}Bi, with description of the radioactivity emissions of ^{212}Pb and its daughters. The emissions are expressed in MeV.

unmodified AE1 or different doses of ^{212}Pb-DOTA-AE1. Group 1 received only unmodified AE1 antibody, while groups 2, 3 and 4 received 10, 25 and 40 μCi of ^{212}Pb-DOTA-AE1, respectively.

Radioimmunotherapy of HER2/neu-Expressing Tumors with Lead-212 AE1

In all radioimmunotherapy experiments, we used female athymic nude mice (10–12 wk old). In the initial preliminary therapeutic trial, mice were given i.p. injections of 2×10^6 SK-OV-3 cells. Three days later, groups of mice were given i.p. injections of ^{212}Pb-AE1 at dose levels of 10 and 20 μCi (specific activity, 0.6–1.5 μCi/μg), the control radiolabeled monoclonal antibody (^{212}Pb-anti-Tac) at the same dose levels (specific activity, 1.0–2.5 μCi/μg), unlabeled AE1 (10 μg) or no treatment. In the second trial, animals were inoculated with 2×10^6 SK-OV-3 cells s.c. in the back of the neck. Three days later, groups of five mice were treated i.v. with either ^{212}Pb AE1 or ^{212}Pb-anti-Tac at dose levels of 10 and 20 μCi. Control groups again received either i.v. unlabeled anti-Tac or no treatment. In the third trial, groups of five mice with established small-sized s.c. tumors (studied 14 days after s.c. tumor inoculation when mean tumor volume was 15 mm^3) were treated with the same agents under the same i.v. protocol, whereas in the fourth group animals with larger (mean tumor volume, 146 mm^3) tumors were studied with the same agents as used in trials 2 and 3. The primary end point in the initial two trials was tumor-free survival (time from treatment until tumor occurrence). Data were evaluated according to the methods of Kaplan and Meier (*25*) and Mantel and Haenszel (*26*). For the third and fourth studies, the observed parameter was the change in tumor volume calculated as $(L \times W^2)/2$, where L is length and W is width. The tumor volume for each animal at the time indicated was normalized to the tumor volume of the animal that was present on the day of therapy.

RESULTS

SK-OV-3 Murine Model of a HER2/neu-Expressing Human Solid Tumor Model

In our initial studies, we established a murine tumor model for human HER2/neu-directed alpha particle-emitting radioimmunotherapy using the SK-OV-3 human ovarian tumor cell line. The SK-OV-3 cell line expressed approximately 10^6 HER2/neu receptors per cell, as defined by Scatchard analysis using the ^{125}I-anti-HER2/neu antibody AE1. With this cell line, after administration of 2×10^6 cells, tumor occurrence rate was over 95%, with a median time to tumor appearance of 8 days after s.c. injection, and the occurrence rate was 60%, with a median time to appearance of 10–13 days after i.p. inoculation. The s.c. administered tumor remained localized, but grew to a diameter of 1 cm in 15–71 days (mean, 38 days) and to a diameter of 2 cm in slightly over 100 days.

To define the radiosensitivity of SK-OV-3 cells and to provide a baseline for comparison with other tumor models used to evaluate radioimmunotherapeutic agents, we quantitated tritiated thymidine uptake and defined the percentage of remaining cells still capable of colony formation after external irradiation of SK-OV-3 cells at different dose levels (0–10,000 rad). A dose of 520 rad reduced the colony-forming ability of irradiated cells to 2% of that observed with nonirradiated cells.

Biological Activity of DOTA-AE1

To be effective in radioimmunotherapy of HER2/neu-expressing tumors, the chelating agent used to link the radionuclide to the AE1 antibody should not alter the specificity or binding affinity of the monoclonal antibody to its antigenic target. The binding integrity of DOTA-AE1 was assessed by comparing it to unlabeled AE1 in a competitive binding assay (Fig. 1). In this assay, SK-OV-3 cells were incubated with a nonsaturating amount of ^{125}I-AE1, along with increasing quantities of either unmodified or DOTA-AE1. The chelated antibody manifested identical binding activity as unmodified antibody when evaluated in this competition assay.

In Vivo Pharmacokinetics and Tissue Distribution of Radiolabeled DOTA-AE1

The stability of a ^{203}Pb-labeled monoclonal antibody using DOTA as a chelating agent has been examined previously and shown to be stable under in vivo conditions (*24*). To test the in vivo stability of the particular DOTA-AE1 antibody used in this study, we used ^{88}Y rather than ^{212}Pb, in light of its longer physical half-life and gamma emission. The yttrium–DOTA complex has been shown to be and is also generally accepted to be inert and stable in vitro and in vivo, thus providing an unequivocal biodistribution standard for a radiolabeled antibody using a chelated metal. We and others have noted that the indium complex formed with DOTA was not identical with the yttrium complex and, therefore, ^{88}Y was used as an accurate tracer isotope. In these pharmacokinetic studies, animals with established s.c. SK-OV-3 tumors (~1 g) were given i.v. injections of ^{88}Y-DOTA-AE1 (2 μCi per mouse), serial blood samples were collected after injection and the fraction of the dose remaining in the plasma was determined. The terminal

TABLE 1
Tissue Distribution of Yttrium-88-AE1 and Yttrium-88-Anti-Tac in Percentage of Injected Dose per Gram of Tissue

	Yttrium-88-AE1 specific antibody					Yttrium-88-anti-Tac control antibody	Specific-to-control ratio of incorporation
	2 hr	6 hr	10 hr	24 hr	48 hr	24 hr	24 hr
SK-OV-3 tumor	3.70 ± 0.88	7.70 ± 0.28	10.35 ± 0.57	26.05 ± 6.17	24.67 ± 3.88	6.40 ± 2.35	4.07
Blood	35.57 ± 5.05	30.18 ± 4.13	26.65 ± 4.34	23.81 ± 6.01	17.38 ± 3.19	21.50 ± 0.84	1.11
Liver	11.57 ± 0.34	9.90 ± 1.10	8.42 ± 1.30	9.15 ± 1.33	6.42 ± 0.73	6.56 ± 0.00	1.39
Spleen	6.45 ± 0.63	6.02 ± 0.53	5.70 ± 1.25	6.13 ± 1.46	5.45 ± 0.42	6.07 ± 0.20	1.01
Kidney	9.57 ± 0.35	8.44 ± 1.02	7.25 ± 0.53	8.13 ± 1.00	5.79 ± 0.35	6.08 ± 0.17	1.34
Intestine	2.88 ± 0.10	2.58 ± 0.14	2.26 ± 0.00	2.42 ± 0.36	1.83 ± 0.26	2.24 ± 0.19	1.08
Heart	13.82 ± 0.56	10.54 ± 0.48	9.17 ± 1.72	10.80 ± 3.05	8.80 ± 0.72	8.50 ± 1.01	1.27
Lung	11.48 ± 0.70	10.38 ± 0.69	8.50 ± 0.81	10.53 ± 3.06	6.98 ± 0.62	9.14 ± 0.37	1.15
Femur	2.31 ± 0.20	2.05 ± 0.24	1.90 ± 0.20	2.37 ± 0.44	1.94 ± 0.10	2.25 ± 0.00	1.05

half-life (beta) of these studies was 86 hr; the fraction intravascular was 56% with 33% of the i.v. pool catabolized per day as assessed by the method of Matthews (*27*).

The effective treatment of large established tumors with ^{212}Pb-AE1 is dependent in part on the physical half-life of ^{212}Pb (10.6 hr) and the time required to achieve an optimum tumor-to-normal tissue ratio of radioactivity after i.v. administration. To address this issue, animals with established s.c. SK-OV-3 tumors were given i.v. injections of either tumor-specific antibody (^{88}Y-AE1 or of an irrelevant antibody ^{88}Y-anti-Tac). In Table 1, we show the tissue distribution of ^{88}Y-AE1 and the control antibody ^{88}Y-anti-Tac expressed as a percentage of the injected dose per gram of tissue in these tumor-bearing mice, as well as the ratio of specific-to-control antibody incorporation into different organs 24 hr after the i.v. infusion. The values are expressed as the mean ± s.d. of three mice killed at each time point. Within the first 6 hr, there was essentially no enrichment of AE1 in the SK-OV-3 tumors compared with normal tissues. However, by 24 hr, SK-OV-3 tumors manifested an enrichment of ^{88}Y-DOTA-AE1, yielding tumor-to-normal solid tissue ratios of 2.85–11 to 1. Such enrichment was not observed with the control monoclonal antibody anti-Tac. Furthermore, the ratio of incorporated specific antibody (AE-I) to control antibody (anti-Tac) at 24 hr was 4.07 for the tumor, whereas there was no enrichment (ratios of 1.01–1.39) with the normal organs.

Radioactivity–Toxicity Relationship and Maximum Tolerated Dose of Lead-212-AE1

To define an activity-toxicity relationship for ^{212}Pb-AE1, increasing doses (10, 25 and 40 μCi) of ^{212}Pb-AE1 were administered i.v. into tumor-free athymic nude mice, and the effects on hematological and serum chemistry parameters were defined over a period of 180 days. The activity-limiting toxicity was bone marrow suppression in animals receiving a more than 25-μCi dose of lead-radiolabeled AE1 (Fig. 3). Specifically, all animals receiving the 40-μCi dose of ^{212}Pb-labeled monoclonal antibody died within 6–10 days of therapy, manifesting a mean weight loss of 25.8%. Pathological examination of the tissues revealed significant lymphoid depletion and a marked reduction in the size of the spleen (which, on histological examination, was associated with decreased extramedullary hematopoiesis), an acellular bone marrow and pulmonary congestion. Four of the five animals in this group developed septicemia, with bacterial infections affecting the various tissues. All 16 animals receiving 25- and 10-μCi doses of ^{212}Pb monoclonal antibody i.v. survived, but manifested transient granulocytopenia, lymphocytopenia and modest thrombocytopenia (Fig. 3). The animals receiving the 25-μCi dose developed a nadir in their hematopoietic elements in the period spanning days 4–11, with a leukocyte nadir of 400 cells/μl (normal leukocyte range in mice, 2,600–10,700 μl), lymphocyte levels of approximately 250 (normal lymphocyte range in mice, 1,430–9,940 μl) and thrombocytopenia with a nadir of approximately 170,000 μl (normal platelet count range, 592,000–2.97 × 10^6/μl). The platelet and total white blood cell levels returned to the normal range by day 20, whereas the lymphocyte levels were normal

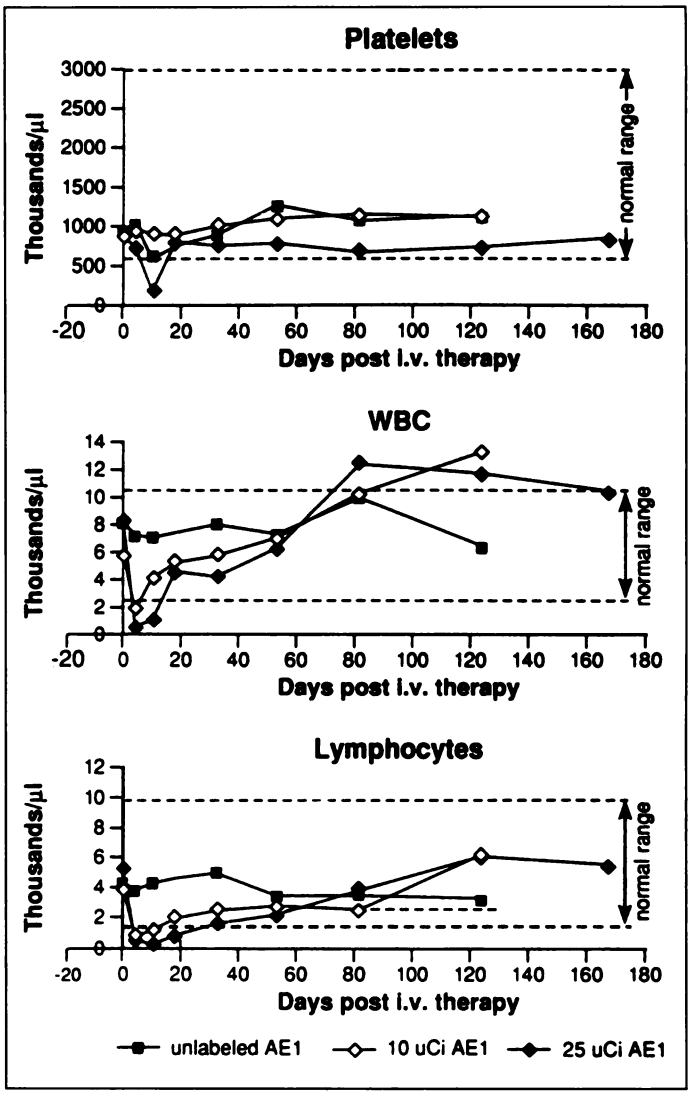

FIGURE 3. The effect of i.v. administered unlabeled AE1 (■), 10 μCi of ^{212}Pb-AE1 (◇) and 25 μCi of ^{212}Pb-AE1 (◆) on platelet, white blood cell (WBC) and lymphocyte levels.

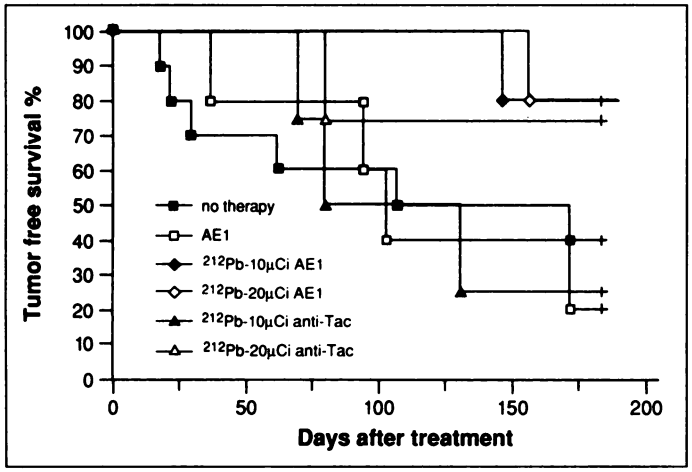

FIGURE 4. Tumor-free survival after i.p. treatment with 20 μCi of ^{212}Pb-AE1 (◇; n = 4), 10 μCi of ^{212}Pb-AE1 (◆; n = 4), 20 μCi of ^{212}Pb-anti-Tac (△; n = 5), 10 μCi of ^{212}Pb-anti-Tac (▲; n = 5), unlabeled AE1 (□; n = 5) or no treatment (■; n = 10) 3 days after i.p. inoculation with 2 × 10^6 SK-OV-3 cells. p_2 = 0.015, 10- and 20-μCi AE1 treatment groups compared with the two control groups not treated with a radiolabeled monoclonal antibody. p_2 = 0.06, comparison of ^{212}Pb-AE1 to ^{212}Pb-anti-Tac groups.

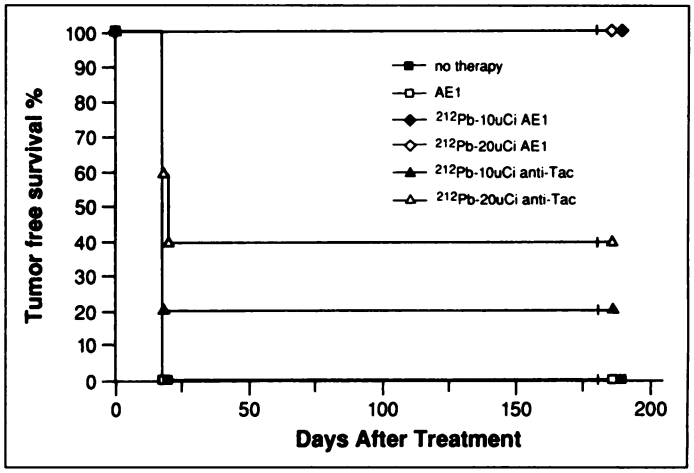

FIGURE 5. Tumor-free survival after i.v. treatment with 20 μCi of ^{212}Pb-AE1 (◇; n = 4), 10 μCi of ^{212}Pb-AE1 (◆; n = 4), 20 μCi of ^{212}Pb-anti-Tac (△; n = 5), 10 μCi of ^{212}Pb-anti-Tac (▲; n = 5), AE1 (□; n = 5) or no therapy (■; n = 10) 3 days after s.c. inoculation with 2 × 10^6 SK-OV-3 cells. p_2 = 0.002, comparison of 10- and 20-μCi AE1 treatment groups with the two control groups not treated with a radioactive monoclonal antibody.

after day 60. Seventy-five percent of the mice receiving 25 μCi of ^{212}Pb-AE1 or ^{212}Pb anti-Tac developed long-term renal toxicity with elevated mean blood urea nitrogen or creatinine. The levels of blood urea nitrogen and creatinine for all surviving mice were 39 and 0.8 mg/dl, respectively, compared with the upper limits of the normal range for mice of 28 and 0.7 mg/dl, respectively.

Lead-212-AE1 Radioimmunotherapy: Therapeutic Efficacy

We first evaluated the therapeutic efficacy of ^{212}Pb-AE1 in an SK-OV-3 tumor model featuring direct access of the therapeutic agent to the tumor-containing compartment. For this group, 10–12-wk-old female nude mice were given i.p. injections of 2 × 10^6 SK-OV-3 cells. Three days later, different groups of five mice each received i.p. administered ^{212}Pb-AE1 at doses of 10 and 20 μCi, the control radiolabeled monoclonal antibody (^{212}Pb-anti-Tac) at the same dose levels, unlabeled AE1 (20 μg) or no treatment (Fig. 4). Lead-212-AE1 treatment resulted in a statistically significant delay in the appearance or prevention of tumor occurrence when mice received 10- and 20-μCi doses; considered together, these mice were compared with the two groups of mice receiving either no treatment or unlabeled AE1 (p_2 = 0.015). In interpreting these exploratory studies, it should be noted that only 60% of the animals in the control group injected i.p. with SK-OV-3 cells developed tumors.

A second group of animals was used to obtain a more meaningful evaluation of the effectiveness of ^{212}Pb-AE1 therapy in the adjuvant setting. In this study trial, nude mice were injected with 2 × 10^6 SK-OV-3 cells s.c. in the back of the neck. Three days later, mice were treated i.v. with ^{212}Pb-AE1 or control ^{212}Pb-anti-Tac at dose levels of 10 and 20 μCi, treated with unlabeled AE1 or received no treatment. The Kaplan–Meier plot depicting tumor-free survival after treatment revealed no differences between the group of animals receiving no therapy (n = 10) and that receiving unlabeled AE1 (n = 5); all animals in both groups developed a tumor by day 20. In contrast, all animals receiving either 10 or 20 μCi of ^{212}Pb-AE1 remained tumor-free throughout the 180-day study period (Fig. 5; p_2 = 0.002, animals receiving low and high doses of ^{212}Pb-AE1 compared with those in the nontreated group). We conclude that this effectiveness in preventing the development of tumors was not due solely to nonspecific irradiation because four of five animals receiving 10 μCi of ^{212}Pb-anti-Tac and three of five receiving 20 μCi of this control radiolabeled monoclonal antibody developed tumors by day 22 (p_2 = 0.003, animals receiving 10 or 20 μCi of AE1 compared with animals receiving the same doses of radiolabeled anti-Tac). The animals surviving after i.p. and s.c. inoculation of the SK-OV-3 tumor were killed on day 180, and the tissues obtained at necropsy were submitted for histological evaluation. At that time, there was no evidence of residual tumor or bone marrow abnormality.

A third trial group of mice with small-sized established s.c. tumors (studied 14 days after tumor inoculation when mean tumor volume was 15 mm^3) was treated with the same agents using the same protocol (Fig. 6). The rate of tumor growth was inhibited in the period after therapy with 20 μCi of ^{212}Pb-specific antibody. However, no complete remissions were observed. A fourth group of animals had large (mean tumor volume, 146 mm^3) s.c. SK-OV-3 tumors when they were

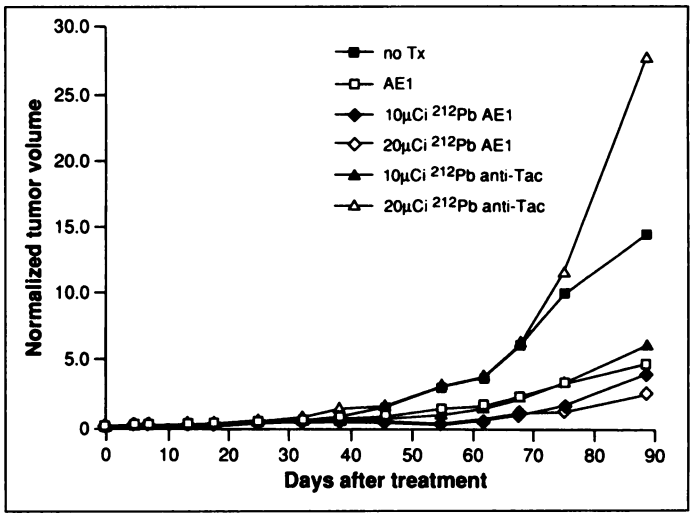

FIGURE 6. Tumor growth of small s.c. SK-OV-3 tumors (mean tumor volume, 15 mm^3) after i.v. treatment with 20 μCi of ^{212}Pb-AE1 (◇), 10 μCi of ^{212}Pb-AE1 (◆), 20 μCi of ^{212}Pb anti-Tac (△), 10 μCi of ^{212}Pb anti-Tac (▲), unlabeled AE1 (□) and no therapy (■). The tumor volume for each animal at the time indicated was normalized to the tumor volume for that animal that was present on the day of treatment. Mean values for the five animals in each group are shown.

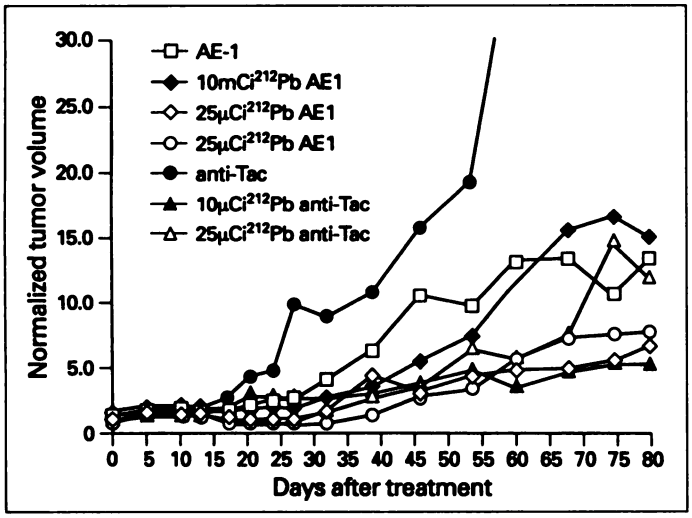

FIGURE 7. Tumor growth of larger (mean tumor volume, 146 mm^3) established s.c. SK-OV-3 tumors after i.v. treatment with 25 μCi of ^{212}Pb-AE1 (◇), 25 μCi of ^{212}Pb-AE1 (○), 10 μCi of ^{212}Pb-AE1 (◆), 25 μCi of ^{212}Pb-anti-Tac (△), 10 μCi of ^{212}Pb-anti-Tac (▲), unlabeled AE1 (□) and unlabeled anti-Tac (●). The tumor volume for each animal at the time indicated was normalized to the tumor volume for that animal that was present on the day of treatment. Mean values for the five animals in each group are shown.

treated with the same agents (Fig. 7). Tumor growth was not inhibited in these mice bearing large SK-OV-3 tumors by the administration of a single doses of 25 μCi of ^{212}Pb-specific antibody as compared to those receiving the nonspecified radiolabeled anti-Tac antibody. In particular, the size of the tumors increased in all treatment groups and there were no partial or complete remissions. Thus, ^{212}Pb-AE1 did not provide effective therapy for large established tumors.

DISCUSSION

The use of monoclonal antibodies armed with toxins or radionuclides to specifically target these cytotoxic agents to tumor cells provides a valuable alternative for cancer therapy. Factors that appear critical in developing an effective radioimmunotherapeutic regime include: the choice of the antigenic target and the selection of the monoclonal antibody; the choice of radionuclide; and the selection of the chelate. A monoclonal antibody is selected, in part, based on the specificity and binding affinity of the antibody to its target. We selected AE1 because of its ability to bind to the HER2/neu oncoprotein, a receptor that is overexpressed in some adenocarcinomas of the breast, ovary, lung and stomach (*7–14*). Lead-212-AE1 produced encouraging therapeutic results in the adjuvant setting when it was used to prevent tumor development. However, this agent did not provide effective therapy for large established tumors. It is possible that the results may be improved by using an alternative antibody to the HER2/neu oncoprotein and a different HER2/neu-expressing tumor model. This view is in accord with recent reports suggesting that an additional feature that may be critical in developing an effective agent for radiolabeled monoclonal antibody treatment is the choice of an antibody that, in its unmodified state, has an antitumor effect, especially one that involves induction of apoptosis in sensitive tumor cells (*28–30*). An antibody that induces apoptosis may work synergistically with protracted low-dose irradiation to kill tumor cells.

When assessed in terms of induction of tyrosine phosphorylation of the HER2/neu receptor-expressing cells, the AE1 antibody used in this study did not stimulate tyrosine phosphorylation of the targeted HER2/neu oncoprotein, nor did it inhibit cell proliferation or induce apoptosis. However, other antibodies are available that act as agonists of tyrosine phosphorylation and inhibitors of tumor cell proliferation and viability (*7–9*). These antibodies will be used in subsequent radiolabeled monoclonal antibody therapeutic models.

A second critical component to consider is the selection of the radionuclide. An appropriate choice of radionuclide would be one that has a short distance of action (e.g., one with a beta or alpha emission) that will, thereby, maintain the antigen specificity of the monoclonal antibody and kill antigen-expressing tumor cells, but will spare distant normal cells. Iodine-131 is by far the most frequently used radionuclide in radioimmunotherapy. However, in most cases, ^{131}I-labeled monoclonal antibodies have been relatively ineffective due to limitations of the radionuclide as a therapeutic agent. In light of the limitations of radioiodine, metallic radionuclides that can be securely linked to antibody may provide a better choice. Antibodies may be armed with beta- or alpha-emitting radionuclides. Beta-emitting radionuclides such as ^{90}Y-labeled monoclonal antibodies can kill nontargeted antigen-nonexpressing tumor cells through a "cross-fire" effect from neighboring antigen-expressing cells that have been targeted by the radiolabeled monoclonal antibody (*3,31*). Alpha-emitting radionuclides may provide other advantages as agents that kill tumor cells without damaging distant normal tissues (*2,4,6,28,29,32*). Radionuclides emitting alpha particles release high-energy emissions (6–9 MeV, at least 5 times greater than beta or gamma emitters) over a short distance (40–80 μm) and are efficient at killing individual target cells without significantly penetrating normal tissues. Alpha-emitting radionuclides available for immunotherapy that are under investigation include ^{212}Bi, ^{213}Bi, ^{211}At and ^{212}Pb (alpha emission from daughter ^{212}Bi). Our previous radioimmunotherapeutic trial using an alpha-emitting radionuclide focused on ^{212}Bi (*32*). Although ^{212}Bi may be useful in adjuvant or leukemia therapy, it appears to be of limited value in the therapy of solid tumors due to the short physical half-life (1 hr) of ^{212}Bi and the time required after i.v. administration to achieve a meaningful tumor-to-normal tissue ratio of radioactivity. However, alternative alpha-emitting radionuclides with longer half-lives are available. In particular, we have chosen ^{212}Pb with a 10.6-hr half-life that is the parent of ^{212}Bi as an alternative. Lead-212 anti-HER2/neu would appear to be useful in settings where a small number of tumor cells are readily accessible to the administered radiolabeled monoclonal antibody. Lead-212 may also be useful for compartmental therapies in hollow viscera or spaces (e.g., peritoneum) where the distribution is rapid. Rotmensch et al. (*6*) administered ^{212}Pb sulfur colloid i.p. to nude mice bearing the human OVCAR-3 ovarian carcinoma. Tumor necrosis with a decrease in ascites was noted, although acute gastrointestinal toxicity developed. Finally, ^{212}Pb may be valuable in strategies such as that proposed by Burrows et al. (*33*), in which cytotoxic agents would be targeted to the vasculature of the tumor.

A third pivotal factor in designing an optimal radioimmunotherapeutic reagent is the choice of the method used to link the radionuclide to the monoclonal antibody. In the case of metals, it is critical that the chelate should not alter the specific binding of a monoclonal antibody to its antigenic target. It should not damage the antibody and thus alter its rate of catabolism or patterns of tissue distribution, and it should hold the radiometal tightly so there is no premature elution of the radioisotope from the monoclonal antibody chelate complex in vivo. The DOTA chelate fulfilled the first two requirements. In particular, chelated antibody manifested identical binding activity as unmodified antibody when assessed in a competitive binding assay. Furthermore, the ^{88}Y-labeled chelated anti-HER2/neu antibody

manifested pharmacokinetics after i.v. administration that were comparable to those of the radioiodinated anti-HER2/neu antibody. However, the DOTA chelate did not fully prevent lead complex dissociation (*24,34–37*). Mirzadeh et al. (*24*) demonstrated that the DOTA parent and daughter complexes were stable. That is, when kinetic studies were performed with ^{203}Pb (II) and ^{206}Bi (III), there was virtually no loss of radiometal from the DOTA-chelated antibody in aqueous solutions at pH 4–10. However, on decay of ^{212}Pb to ^{212}Bi-in the DOTA-chelated antibody, there was a loss of 36% ± 2% of the bismuth from the Bi-DOTA complex (*24*). This loss appeared to be due to the electronic excitation that occurs due to the internal conversion process involved in the transition from radiolead to radiobismuth. Thus, ^{212}Pb-DOTA does not fulfill one of the requirements of an optimal radioimmunotherapeutic agent.

CONCLUSION

Lead-AE1, as an intact antibody, was shown to be of value in the adjuvant setting in preventing the development of tumors when therapy was initiated 3 days after tumor cell injection. The growth of large tumors was not affected. However, in this study, we have not taken full advantage of the radioimmunotherapeutic opportunities provided by a ^{212}Pb-labeled monoclonal antibody. The therapeutic efficacy might be improved by changing the tumor model to one involving a HER2/neu-expressing tumor that is responsive to positive or negative signals mediated through HER2/neu receptors and by choosing an anti-HER2/neu monoclonal antibody that, in its unmodified form, induces tumor cell apoptosis. Such an antibody may work synergistically with protracted low-dose irradiation to kill tumor cells. Finally, ligands should be developed that satisfy the stringent demands placed on a metal chelate system to be used for ^{212}Pb radioimmunotherapy.

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