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Radioimmunotherapy of A431 Xenografted Mice with Pretargeted B3 Antibody-Streptavidin and ^{90}Y-labeled 1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic Acid (DOTA)-Biotin

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ABSTRACT

We investigated the biodistribution of ^{88}Y/^{111}In-labeled 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA)-biotin and therapy with ^{90}Y-labeled DOTA-biotin in tumor-bearing mice after B3-streptavidin antibody conjugate (B3-SA) pretargeting. B3 antibody, recognizing Lewis^y antigen, was conjugated to streptavidin (B3-SA). For pretargeting, 400 μg of the B3-SA was injected i.v. into mice bearing A431 tumor xenografts. After tumor localization of B3-SA, 100 μg of synthetic clearing agent was injected i.v. to clear the unbound B3-SA from the circulation. Four h later, 1 μg of radiolabeled DOTA-biotin was injected i.v. Radioimmunotherapy was performed with doses of 9.25 to 37 MBq of ^{90}Y-labeled DOTA-biotin. As a result, radiolabeled DOTA-biotin cleared rapidly. All of the normal tissues had <2.6% of the injected dose per gram, whereas tumor uptake reached ~15% ID/g. The total tumor uptake of radioactivity remained similar for 96 h or longer. In the first study, the median survival of the control group was 8 days, whereas it increased to >163 days in the 37 MBq ^{90}Y group ($P < 0.005$). In a second therapy group, 7 of 10 mice receiving 37 MBq of ^{90}Y and B3-SA were cured, and remained healthy for >180 days after therapy, compared with control groups, with ≤29.2 days mean survival time ($P < 0.001$). Tumor pretargeting with B3-SA and radiolabeled DOTA-biotin has shown favorable, specific, and fast targeting that has resulted in good tumor responses and, thus, serves as a rationale for human studies with the B3-SA pretargeting approach.

INTRODUCTION

Clinical studies using radiolabeled MAbs[2] for radioimmunotherapy of lymphomas have shown promising results (1). In contrast, studies performed with MAbs directed against epithelial tumors have rarely shown partial or complete remissions (1). Various physiological factors contribute to the limited therapeutic response of this targeting approach using intact MAb (1–3). Approaches are needed that result in faster delivery of higher concentrations of radiolabeled MAbs to tumor with relative sparing of normal tissues.

Several pretargeting methods that dissociate the targeting step from the radioactivity delivery step have been proposed (1, 4–6). Some of the methods are based on the high affinity binding of avidin or streptavidin to biotin (7). A promising pretargeting approach consists of three steps (8, 9): (*a*) the SA conjugate is allowed to target and accumulate in tumor, thus carrying the SA receptor that will later bind the radiolabeled biotin; (*b*) the nontumor bound MAb-SA is cleared from the circulation to prevent it from binding the radiolabeled biotin; (*c*) the radiolabeled biotin is administered i.v. and because of its small size, the radiolabeled biotin extravasates out of the circulation quickly and can bind to the SA on the MAb-SA conjugate that has localized in tumor (8–10).

B3 is an excellent antibody to consider for pretargeting applications (11). Biodistribution and radioimmunotherapy of ^{111}In/^{90}Y-radiolabeled B3 antibody has shown good tumor localization, although at the maximal tolerated doses tumor responses were not observed (12). Using the pretargeting approach, it is likely that higher doses of ^{90}Y could be delivered to tumor compared with normal tissues and thus increase the therapeutic index. In this report, we performed a biodistribution study using ^{111}In- and ^{88}Y-labeled DOTA-biotin after pretargeting with B3-SA, as well as a radioimmunotherapy trial with ^{90}Y-labeled DOTA-biotin.

MATERIALS AND METHODS

Pretargeting Reagents. B3 is a murine IgG1k MAb directed at Lewis^y and polyfucosylated-Lewis^x (11). B3 was conjugated to SA by NeoRx by incubating for ~1 h with succinimidyl 4-(*N*-maleimido-methyl) cyclohexane-1-carboxylate (10). To confirm that the conjugated SA to B3 was still functional, excess B3-SA was incubated with ^{111}In-labeled DOTA-biotin, and instant thin-layer chromatography, using paper chromatography developed with normal saline, was performed and showed a bound fraction >99%.

The sCA provided by NeoRx consists of a bifunctional moiety with multiple *N*-acetyl-galactosamine residues linked to biotin (M_r 8651; Ref. 13). When sCA was injected to mice ($n = 5$) that had received ^{125}I B3-SA, a mean drop of 90% of the circulating ^{125}I was observed.

Biotinidase-resistant DOTA-biotin (M_r 900) was prepared by NeoRx corporation as described previously and labeled with either ^{111}In, ^{88}Y, or ^{90}Y (14).

Radiolabeling. Unmodified B3 and B3-SA were labeled with 4-[^{125}I]iodobenzoate (15) at a specific activity of 37 kBq/μg for biodistribution studies. B3 conjugated with 2-(*p*-isothiocyanato benzyl)-cyclohexyl-diethylenetriamine-pentaacetic acid (CHX-A″-referred to as CHX) was also labeled with ^{111}In at a specific activity of 37 kBq/μg for biodistribution study as described previously (16). Biotinidase-resistant DOTA-biotin was labeled with ^{111}In or ^{88}Y at a specific activity of 0.37 to 1.11 MBq/μg for biodistribution and with ^{90}Y at a specific activity of 37 MBq/μg for therapy (10). These radiolabeled DOTA-biotin reagents consistently bound >99% to B3-SA.

Immunoreactivity Assay. A431, a human epidermoid carcinoma cell line that expresses the antigen recognized by B3, was used for immunoreactivity determination (16). The immunoreactivity of ^{125}I B3-SA (68–73%) was in the range of that of ^{125}I B3 (70–77%). A competition assay performed using 10^5 A431 cells incubated with ^{125}I B3 and increasing doses of unlabeled B3 or B3-SA showed overlapping curves (data not shown). The IC_{50} was 89 nM and 95 nM for B3 and B3-SA, respectively (GraphPad Prism, San Diego, CA).

Tumor Model. Female athymic mice (*nu/nu*) were inoculated s.c. with A431 cells. Animal experiments were performed under an NIH Animal Care and Use Committee approved protocol. Biodistribution or therapy studies were performed when xenografted tumors typically reached a maximum diameter of ~0.5 cm (0.11–0.31 g). Mice were killed when tumor size reached >2 cm in the longest diameter, tumor was ulcerated, or excessive weight loss (>25%) was noted, according to the protocol guidelines. Seven days before pretarget-

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[2] The abbreviations used are: MAb, monoclonal antibody; %ID/g, injected dose per gram; SA, streptavidin; sCA, synthetic clearing agent; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; PSL, photoluminescence stimulated unit; HAT-SA, humanized anti-Tac monoclonal antibody-streptavidin; CBC, complete blood count.

ing, the mice were given a biotin-free diet to reduce their endogenous biotin level (Biotin Deficient Purina Diet 5836C; Purina Mills, Richmond, IN). One week of this diet in BALB/c mice ($n = 3$ per group) caused their biotin level in serum to drop from a baseline of 15.0 ± 5.2 ng/ml to 4.77 ± 1.12 ng/ml (Vitamin Diagnostics, Inc., Cliffwood Beach, NJ).

Optimization of B3-SA Dose. To evaluate the effect of MAb mass on tumor targeting and thus determine the optimal MAb dose for pretargeting, biodistribution studies were performed with ^{111}In-CHX-B3. B3 was used rather than B3-SA because studies in tumor bearing mice showed a similar biodistribution in most tissues of ^{111}In-B3 and B3-SA (data not shown), and because the quantity of B3-SA was limited. Tumor-bearing mice were injected i.v. with 0.185 MBq of ^{111}In-CHX-B3. The amount of B3 was adjusted by adding unlabeled, unconjugated B3 in doses of 5, 50, 100, 200, 400, and 800 μg. At each dose level 5 mice were sacrificed 48 h after injection. Two additional groups were sacrificed at 24 and 72 h after receiving 400 μg to better characterize the kinetics of the dose finally selected for the therapy trial.

To evaluate the effect of dose on gross heterogeneity of distribution within the tumor, autoradiography was performed after injection of the ^{111}In-CHX-B3. The autoradiography was performed using a BAS-1500 Fujifilm Bio-imaging analyzer (Fuji Medical Systems, Inc.). The slides were exposed to the imaging plate, and developed and analyzed according to the manufacturer's procedure. The autoradiograms were inspected visually, and areas of interest were drawn around the individual tumor autoradiogram, and the mean and SD of the counts were determined in the manufacture's PSLs.

^{111}In-and/or ^{88}Y-labeled DOTA-Biotin Biodistribution. Tumor-bearing mice were injected i.v. with 400 μg (1.91 nmol) of B3-SA for pretargeting. After 24 h were allowed for distribution, 100 μg (11.56 nmol) of sCA was injected i.v. to clear circulating B3-SA from the blood. Four h after injection of the sCA, 1 μg (1.11 nmol) of ^{111}In-labeled DOTA-biotin was injected i.v. Groups of 5 mice were sacrificed at 2, 24, 48, 96, and 168 h after injection. In a separate experiment, dual-isotope injection of the ^{111}In- and ^{88}Y-labeled DOTA-biotin was given to mice to allow direct comparison of both reagents at 2 and 96 h.

Dosimetry. The mean %ID/g of ^{111}In in tumor and tissues at the various time points up to 7 days was used to obtain the area under the curve of the exposure for ^{90}Y using trapezoidal integration. From that point on, the activity in tumor was extrapolated, assuming it cleared with the estimated terminal half-life of the clearance curve (GraphPad Prism). ^{90}Y radiation dosimetry was then calculated based on biodistribution data using the medical internal radiation dose method (17) and adjusted for organ size using the "Nodule Module" of MIRDOSE 3 (18). The data from our previous study of ^{111}In B3 (16) was analyzed in the same way and compared with that of ^{90}Y-labeled DOTA-biotin.

Therapy. Radioimmunotherapy was performed in tumor-bearing mice by pretargeting ^{90}Y-labeled DOTA-biotin after allowing for localization of B3-SA and clearing of the blood as described above. Groups of mice ($n = 5$) were treated in a dose escalation trial with 9.25, 18.5, and 37 MBq of ^{90}Y-labeled DOTA-biotin and compared with a no-treatment control group. In an additional experiment, a group of 10 mice was treated with 37 MBq ^{90}Y-labeled DOTA-biotin after the same pretargeting approach with B3-SA and compared with 3 control groups: (*a*) no treatment; (*b*) radioactivity-free group receiving B3-SA, clearing agent, and nonradioactive DOTA-biotin; and (*c*) a nonspecific MAb consisting of HAT-SA, directed against the interleukin 2 receptor, which is not expressed on A431 cells and was pretargeted followed by 100 μg sCA and 37 MBq ^{90}Y-labeled DOTA-biotin (19).

Response to Therapy and Toxicity Assessment. Tumor growth was monitored twice a week for 3 weeks after treatment and then once per week. A digital caliper was used to measure the tumor in two orthogonal dimensions. The volume was calculated using the formula (long dimension) × (short dimension)^2/2.

To determine hematological toxicity, 20 μl of blood was collected by bleeding the tail veins using an EDTA-coated capillary tube and diluted to 250 μl with PBS ($n = 5$/group). WBC, lymphocytes, RBC, and platelet count were performed before therapy and at weekly intervals thereafter. Serum was obtained at the time of sacrifice, and liver (total bilirubin, alk phosphatase, and albumin) and kidney (blood urea nitrogen and creatinine) function test were done.

Histological analysis was performed in 3 mice in each of the groups (control, 9.2 MBq, 18.5 MBq, and 37 MBq of the ^{90}Y-labeled DOTA-biotin-B3-SA, and the 37 MBq ^{90}Y-labeled DOTA-biotin-HAT-SA group). Tissues analyzed included the kidney and liver, and in some animals bone and bone marrow were also fixed in 10% buffered formalin for pathological studies by a veterinary pathologist.

Statistics. Statistical analysis was performed using ANOVA for multiple groups and Student's *t* test for unpaired data between two groups. Survival analysis was based on the Kaplan-Meier product limit, and groups were compared using the log-rank test (GraphPad Prism).

RESULTS

Biodistribution Study. The concentration of ^{111}In-CHX-B3 and ^{111}In-B3-SA in tumor was similar, with concentrations of 13.89 ± 3.16 and 10.33 ± 2.20%ID/g at 24 h, respectively ($P = 0.077$). The fraction of administered ^{111}In-CHX-B3 (%ID/g) in tumor remained similar, with doses ranging from 5 to 400 μg (Fig. 1*A*). Although the %ID/g of radioactivity dropped with the 800 μg dose, the absolute tumor-bound B3 still increased. The tumor uptake of 400 μg was near maximal at 24 h (21.8 ± 2.1%ID/g) and remained grossly unchanged for 24 h through 72 h after injection ($P = 0.74$; Fig. 1*B*). On autoradiography no gross differences in tracer heterogeneity were noted visually in the tumors. The mean activity value and SD obtained from the region of interest analyses over the autoradiographs of tumors, which are a measure of the heterogeneity of the intensity per pixel, were 120 ± 23 PSL units, 164 ± 41 PSL units, 165 ± 62 PSL units, 140 ± 36 PSL units, 116 ± 19 PSL units, and 158 ± 37 PSL units for the 5, 50, 100, 200, 400, and 800 μg antibody doses, respectively (ANOVA, $P = 0.197$).

^{111}In- and ^{88}Y-labeled DOTA-biotin cleared rapidly with >70% of the administered dose excreted by 2 h after injection. The biodistribution of ^{111}In-labeled DOTA-biotin showed rapid and high accumulation in tumor, with 15.8 ± 0.4%ID/g at 2 h after injection, whereas all of the other organs had <2.6%ID/g (Fig. 2). Therefore, the tumor:nontumor targeting ratios at 2 h were high: 6.8 ± 2.8, 12.2 ± 4.5, 8.1 ± 0.8, and 25.9 ± 11.5 for blood, liver, kidney, and bone, respectively. At 24 h the tumor:nontumor ratios were 8.5 ± 3.7, 11.1 ± 4.8, and 5.5 ± 0.5 for blood, liver, and kidney, respectively, whereas they were only 0.88 ± 0.11, 0.34 ± 0.04, and 0.37 ± 0.04 for the non-pretargeted MAb. The %ID/g in tumor peaked at the earliest time point sampled and then decreased over time (Fig. 2). This decrease in %ID/g was almost entirely accounted for by the increasing tumor size from a mean of 0.21 g to 1.03 g over the 7 days (Fig. 2, *inset*).

The biodistribution of the coinfused ^{88}Y- and ^{111}In-labeled DOTA-biotin in tumor-bearing mice showed a similar pattern at 2 h and 96 h postinjection except in the lung, spleen, and bone at 2 h, where the ^{111}In-labeled DOTA-biotin was found in higher concentrations (data not shown).

Radiation Dosimetry. On the basis of ^{111}In-CHX-B3 and ^{111}In-labeled DOTA-biotin biodistribution data ^{90}Y-CHX-B3 and pretargeted ^{90}Y-labeled DOTA-biotin radiation dosimetry for tumor was calculated as 196 Gy/37MBq and 72 Gy/37MBq, respectively, assuming a 1-g tumor that does not increase in size during the therapy and correcting for absorption fraction. The radiation dose to tumor was higher for directly labeled B3. Nevertheless, the expected maximum tolerated dose for ^{90}Y B3 (nonpretargeted) is likely to be in the range of 7.4–11.1 MBq based on prior studies (10, 20). Whereas 37 MBq ^{90}Y could generally be delivered with the pretargeting. The tumor: blood ratios were 2.4 for ^{111}In B3 and 9.3 for pretargeted ^{111}In-labeled DOTA-biotin, respectively.

Therapy Study. Effective and specific therapeutic responses were observed in tumor-bearing mice treated with ^{90}Y-labeled DOTA-biotin after pretargeting with B3-SA. A431 tumors in the no-treatment control group grew rapidly, from 0.2 cm^3 to ~2 cm^3 in <2 weeks

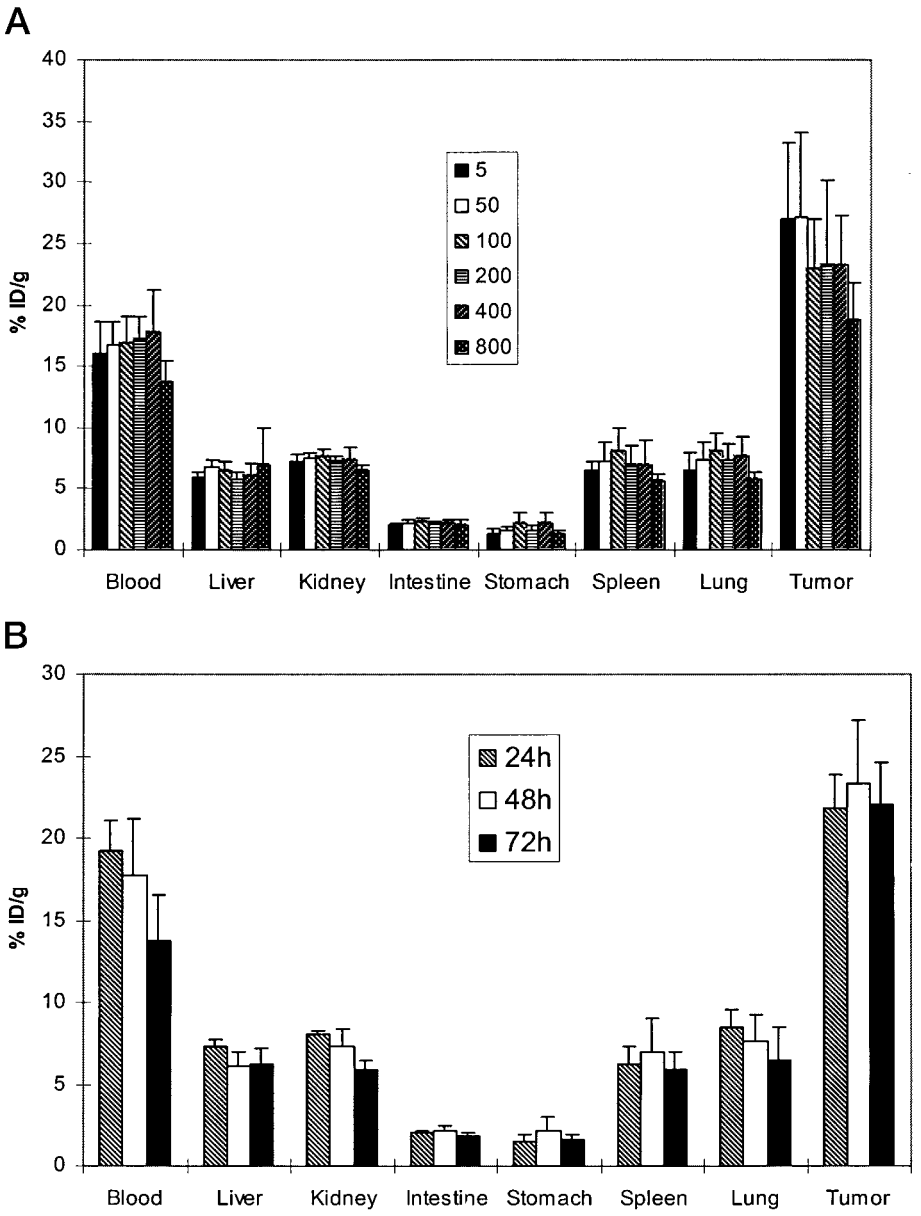

Fig. 1. The biodistribution of ^{111}In-CHX-B3 in groups of mice ($n = 5$) bearing A431 tumor xenografts was evaluated. *A*, the effect of various doses of B3 ranging from 5 to 800 μg on its 24 h biodistribution expressed as the mean %ID/g; *bars*, $\pm$SD. *B*, the biodistribution at various times after i.v. injection of 400 μg of ^{111}In-CHX-B3 in A431 tumor-bearing mice. The data are expressed as mean %ID/g; *bars*, $\pm$SD.

(Fig. 3, *Group 1*), and these animals were sacrificed according to our animal protocol. Escalating doses of 9.25, 18.5, and 37 MBq ^{90}Y-labeled DOTA-biotin with B3-SA pretargeting showed a dose-related response in tumor size (Fig. 3, *Group 2, 3, 4*, respectively). The median duration of survival of the control group was 7 days; it increased to 18, 28, and >180 days in the groups receiving 9.25, 18.5, and 37 MBq of ^{90}Y-labeled DOTA-biotin, respectively. Four of the 5 mice in group 4 and 1 in group 3 were cured and remained healthy for >180 days after therapy, at which time they were sacrificed electively for histology, CBC, liver, and kidney chemistries.

In the second therapy experiment (Fig. 4), a group of mice that received all of the pretargeting steps but no radioactivity showed no response; their tumor growth was similar to the no-treatment control (10 *versus* 12 days median survival, respectively). The mice receiving nonspecific HAT-SA pretargeting and 37 MBq of ^{90}Y-labeled DOTA-biotin showed delayed tumor growth and significantly prolonged survival (24 days) compared with the control group, but complete resolution of tumor was never observed. The mice died or were sacrificed with residual tumor. In the group treated with ^{90}Y-labeled DOTA-biotin after pretargeting with B3-SA, tumor growth was delayed, and 8 of the 10 mice became tumor-free. Median survival was prolonged to >180 days, significantly longer than the other three groups ($P < 0.0001$). Seven of the 10 mice from this group remained healthy for >180 days after therapy and at sacrifice had no visual evidence of tumor. Whereas, of the 3 mice that died, 1 mouse was sacrificed because of tumor progression, 1 mouse with tumor and 1 mouse without tumor died unexpectedly of unknown cause. No autopsy was available on these mice.

All of the mice receiving up to 37 MBq of ^{90}Y therapy survived without radiation-related lethality for 2 weeks. One mouse receiving 18.5 MBq of ^{90}Y during the first therapy study died of a skin disease that had been present in the colony. After therapy, body weights of the nonradioactive control groups increased up to the time of sacrifice because of their large tumors. The weight of the group treated with B3-SA and ^{90}Y-labeled DOTA-biotin showed a mean drop of 6.6% but returned to baseline by 2 weeks. In contrast, the ^{90}Y-labeled

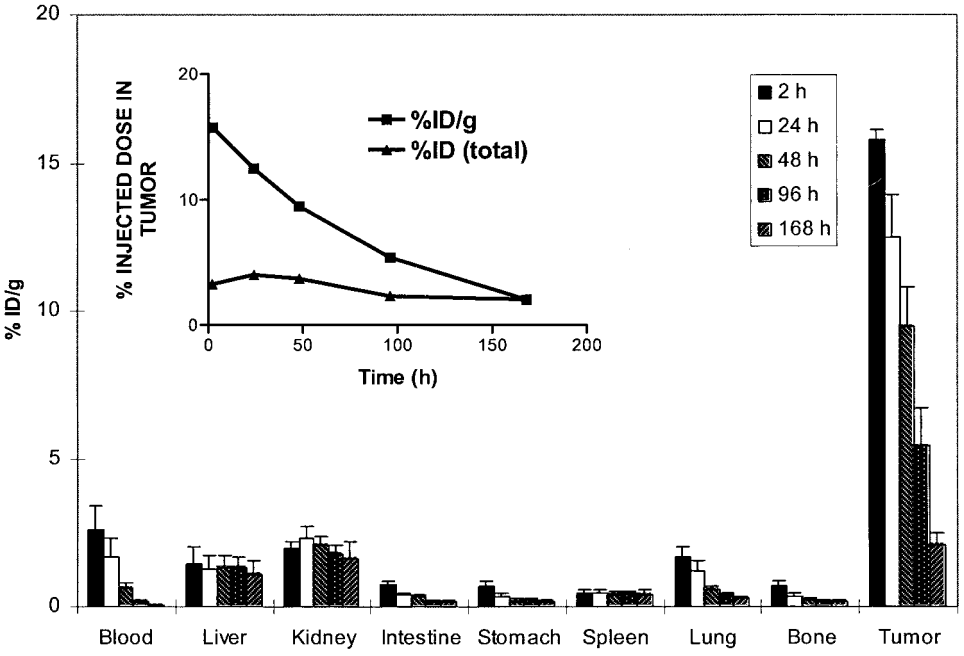

Fig. 2. Biodistribution of pretargeted ^{111}In-labeled DOTA-biotin in mice bearing A431 tumor xenografts that received 400 μg of B3-SA followed by 100 μg sCA clearing agent. Values represent the mean %ID/g; *bars*, ±SD. The insert compares the mean %ID/g tumor and the mean total %ID; *bars*, ±SD (%ID = %ID/g ∗ g tumor weight).

DOTA-biotin group pretargeted with nonspecific HAT-SA had an initial mean drop of 9.6% of their weight and recovered by 21 days, but thereafter the mice progressively weakened and continued to lose weight until all had died or were sacrificed by 41 days. The drops in weight for both ^{90}Y-treated groups were significantly different from each other ($P < 0.02$).

Although only a few animals ($n = 3$) had CBC at each therapy dose, comparison of hematology results in the therapy groups showed that there were no significant differences in platelet and RBC counts between the different groups ($P > 0.12$), with the nadirs occurring at ~2 weeks. Nevertheless, a significant decrease in WBC and lymphocyte counts was observed 1–2 weeks after therapy in the 37 MBq-treated groups ($P < 0.007$), with the nadir occurring at ~2 weeks and recovery by 3 weeks. This represented decreases of 79% and 94% for WBC and lymphocyte count, respectively. Liver and kidney function chemistry performed at the time of sacrifice was no different between nonradioactivity receiving controls or ^{90}Y pretargeted groups.

Histological analysis of the liver and kidney of mice receiving the 37 MBq of ^{90}Y-labeled DOTA-biotin-B3-SA showed similar finding as those of the control group that did not receive radioactivity. In addition, the bone and bone marrow of the mice receiving 37 MBq of ^{90}Y-labeled DOTA-biotin-B3-SA were histologically normal (performed 180 days after therapy). The mice receiving 37 MBq of ^{90}Y-labeled DOTA-biotin-HAT-SA showed similar findings as the control animals and did not have any histological findings in the kidney or liver to explain their toxicity.

DISCUSSION

The pretargeting approach based on SA-biotin depends on the successful delivery and function of three reagents. In this study we showed sufficient concentration of B3-SA to serve as an effective binding receptor for the ^{111}In/^{90}Y-labeled DOTA-biotin. In this study, the %ID/g of the MAb in tumor was shown to be similar for doses of 5–400 μg, whereas the total amount delivered continued to increase. Autoradiography showed that heterogeneity in tumor distribution was not grossly decreased at the higher antibody dose levels. There was some evidence of partial saturation of uptake at the 800 μg dose, as evidenced by the lack of uptake linearity at this higher dose. Our decision to use 400 μg was based on: (*a*) some evidence of saturation at the higher doses that resulted in lower tumor:normal organ uptake ratios; (*b*) very large doses of B3-SA that may be more difficult to clear from the blood and may result in higher tissue background; and (*c*) the results of these experiments, which indicated that the 400 μg dose level resulted in good tumor targeting.

The clearing step and administration of radiolabeled biotin at 24 h after giving B3-SA was chosen because near-peak antibody tumor levels were achieved by this time (Fig. 1*B*). In addition, studies reported previously indicated that despite the low biotin diet, endogenous biotin fills additional binding sites of MAb-SA conjugate at 48 h as compared with 24 h, which decreases the binding sites for the subsequently administered radiolabeled biotin. The doses of sCA and DOTA-biotin we used were effective, as gauged by the high tumor concentration of radiolabeled DOTA-biotin and its fast clearance from the blood (Figs. 2). The dose of these two reagents was based on that shown previously to be effective in other studies (10). These two reagents are "universal" or independent of what MAb-SA conjugate is used, and the doses required for therapy are unlikely to be greatly dependent on what tumor antibody system is used.

Our studies showed that ^{111}In- or ^{88}Y-labeled DOTA-biotin was delivered in similar concentrations to tumor and normal organs. Thus, this similarity allowed us to make dosimetry estimates for ^{90}Y based on ^{111}In biodistribution. The pretargeting approach resulted in rapid delivery of high doses of radiation to the tumor. The calculated dose delivered to the tumor is likely to be an underestimate, because in our pretargeted animals, the tumor size increased, resulting in decreasing %ID/g. If these animals had received therapy, their tumors would not have continued to grow and the radiation dose would have been much higher. ^{90}Y B3 dosimetry extrapolated from previous biodistribution studies suggested that high radiation doses to tumor could be delivered and that much higher doses to normal tissues on an equivalent MBq basis would also be delivered compared with the pretargeting approach. Although the dose-limiting toxicity from ^{90}Y B3 (not

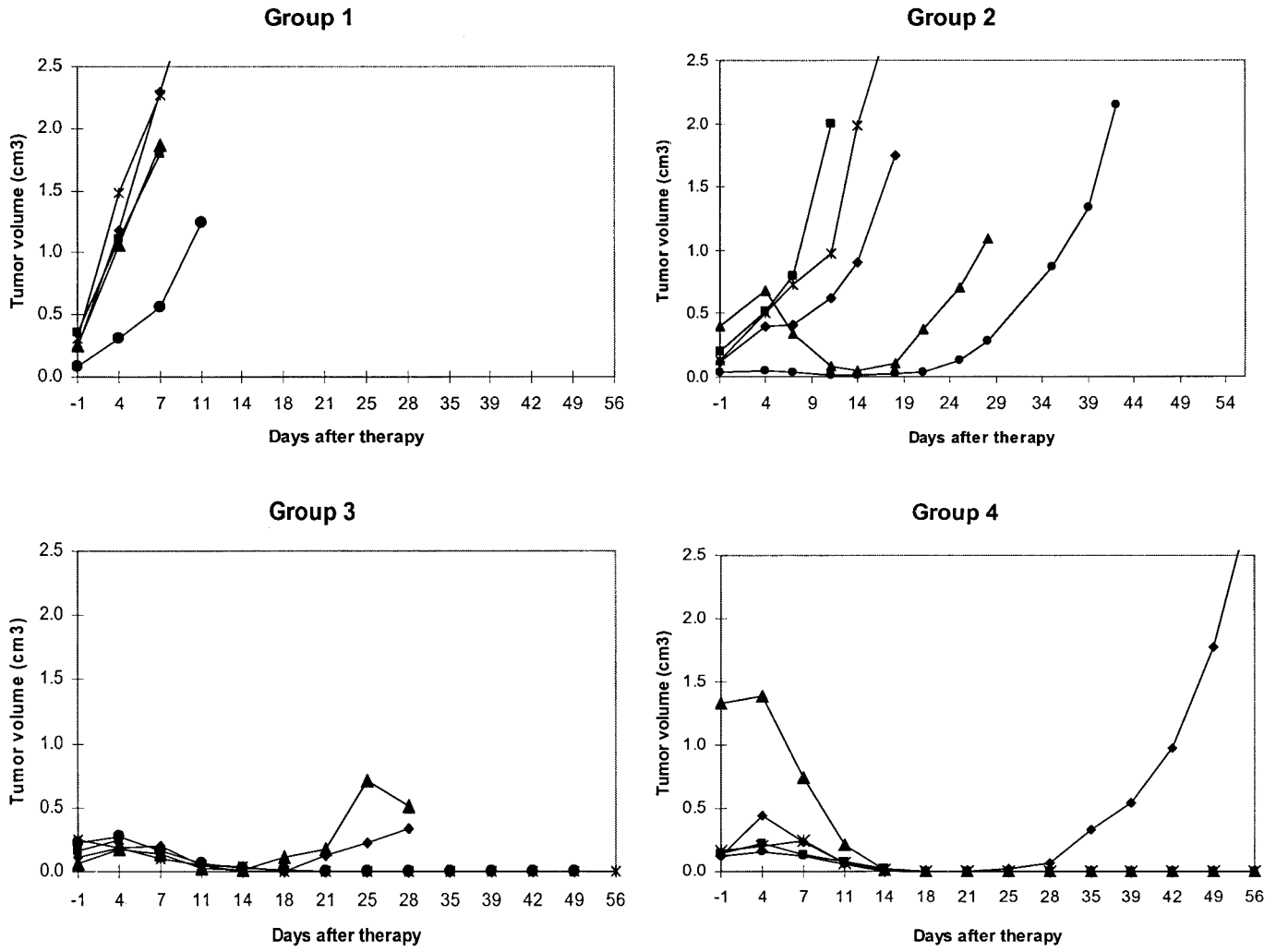

Fig. 3. Response to radioimmunotherapy. Tumor volume changes over time in control mice or mice receiving escalating doses of pretargeted ^{90}Y-labeled DOTA-biotin after receiving 400 μg B3-SA followed by sCA (100 μg). Group 1 is control without any treatment; Groups 2, 3, and 4 received 9.25, 18.5, and 37 MBq of ^{90}Y-labeled DOTA-biotin, respectively. Each symbol represents a single mouse in the group (n = 5 mice/group).

pretargeted) was not evaluated in this study, works by others suggest that the maximal tolerated dose by nude mice was in the range of 7.4–11.1 MBq (10, 20). These lower tolerated doses would significantly limit the maximal radiation dose to tumor as compared with the pretargeted approach, where higher pretargeted amounts of ^{90}Y were possible. Our dosimetry estimates for ^{90}Y B3 on a per MBq basis were in the same range reported by Axworthy *et al.* (10) for nonpretargeted MAb NR-LU-10. This is because of similar peak concentration and kinetics in tumor for their MAb (29.9%ID/g) and ours (26.3%ID/g) at 24 h. In contrast, the peak dose observed in this study from pretargeted radiolabeled DOTA-biotin was lower than the 34.9%ID/g observed previously with NR-LU-10-SA antibody (10). Given the fast clearance of the DOTA-biotin through the kidneys, the ratios of tumor:blood and tumor:bone marrow are more favorable than when ^{90}Y B3 is given without pretargeting. The average tumor:blood area under the curve ratio in our pretargeting studies was 9.3, which compares favorably with the 2.4 ratio seen with ^{90}Y directly conjugated to B3.

Significant decreases of WBC and lymphocyte counts were observed 1–2 weeks after therapy. The platelet counts in the treated and control groups failed to show any significant changes. These results are in line with those reported previously with NR-LU-10-SA, where rapid targeting was observed and where dose-limiting toxicity was not observed at doses of 29.6 MBq (800 μCi). The limited toxicity to the marrow was most likely attributable to the rapid clearing of the unbound ^{90}Y-labeled DOTA-biotin through the kidney, such that with the long physical half-life of ^{90}Y this would not have given a large dose to the whole body, bone marrow, or kidney. Evidence of toxicity was observed when pretargeted ^{90}Y-labeled DOTA-biotin was used with HAT-SA where the mice died or were sacrificed because of tumor ulceration or significant body weight loss within 41 days. Additional studies will be required to determine the cause of toxicity in this control group.

Whereas preclinical animal studies are useful and necessary to help optimize delivery strategies and show proof of principle, use in humans remains the final goal. Given the favorable targeting seen previously *in vivo* with ^{111}In-CHX-B3, the findings in this pretargeting study encourage us to pursue this system in humans (12).

In summary, B3-SA using a three-step pretargeting approach allows rapid and specific delivery of radionuclides to tumor xenografts. High tumor uptake with fast clearance from the vasculature and low nontumor uptake resulted in high tumor doses and good radioimmunotherapeutic responses. These findings support the use of this reagent for a clinical trial in patients.

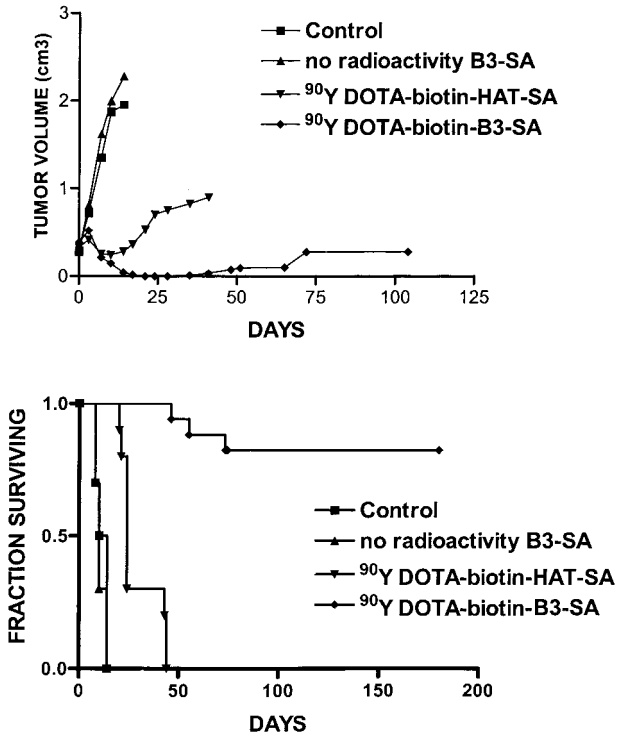

Fig. 4. Response to radioimmunotherapy in mice (n = 10) with A431 tumor xenografts. Four groups were studied: a no-treatment control; a group receiving B3-SA pretargeting (400 μg), sCA (100 μg), and DOTA-biotin but no ^{90}Y; a group receiving 37 MBq of pretargeted ^{90}Y-labeled DOTA-biotin after pretargeting steps with B3-SA and sCA clearing of blood; a group receiving 37 MBq of pretargeted ^{90}Y-labeled DOTA-biotin after all three pretargeting steps with the nonspecific HAT-SA and sCA. *Top panel*, the mean tumor volumes of all surviving mice are shown. Note that follow-up is shorter for the three control groups because of the need to kill the mice when their tumors grew too large. If mice died or were killed early, their last measured tumor volume was used from that point forward and contributed to the mean. *Bottom panel*, the Kaplan-Meier survival curve of mice treated in this second therapy experiment is shown. Note that the control group (no treatment) and the B3-SA group without radioactivity survival curves are almost indistinguishable.

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