Biodistributions, Myelosuppression and Toxicities in Mice Treated with an Anti-CD45 Antibody Labeled with the α-Emitting Radionuclides Bismuth-213 or Astatine-211

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Abstract

We previously investigated the potential of targeted radiotherapy using a bismuth-213-labeled anti-CD45 antibody to replace total body irradiation as conditioning for hematopoietic cell transplantation in a canine model. While this approach allowed sustained marrow engraftment, limited availability, high cost and short half-life of bismuth-213 induced us to investigate an alternative α-emitting radionuclide, astatine-211, for the same application. Biodistribution and toxicity studies were conducted with conjugates of the anti-murine CD45 antibody 30F11 with either bismuth-213 or astatine-211. Mice were injected with 2−50 μCi on 10 μg or 20 μCi on 2 or 40 μg 30F11 conjugate. Biodistribution studies showed that the spleen contained the highest concentration of radioactivity, ranging from 167±23 to 417±109 % injected dose/gram (%ID/g) after injection of the astatine-211 conjugate and 45±9 to 166±11 %ID/g after injection of the bismuth-213 conjugate. The higher concentrations observed for astatine-211-labeled 30F11 were due to its longer half-life, which permitted better localization of isotope to the spleen before decay. Astatine-211 was more effective at producing myelosuppression for the same quantity of injected radioactivity. All mice injected with 20 or 50 μCi astatine-211 but none with the same quantities of bismuth-213 had lethal myeloablation. Severe reversible acute hepatic toxicity occurred with 50 μCi bismuth-213, but not with lower doses of bismuth-213 or with any dose of astatine-211. No renal toxicity occurred with either radionuclide. The data suggest that smaller quantities of astatine-211-labeled anti-CD45 antibody are sufficient to achieve myelosuppression and myeloablation with less non-hematological toxicity compared with bismuth-213-labeled antibody.

Keywords

allogeneic hematopoietic cell transplantation (HCT); transplant conditioning; radioimmunotherapy; astatine-211 (^{211}At); bismuth-213 (^{213}Bi)

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a curative modality for patients with various malignant and non-malignant hematopoietic diseases. Recently, to reduce late toxicities from total body γ-irradiation (TBI) while increasing specificity and efficacy, monoclonal antibodies (MAb) labeled with β-emitting radionuclides, such as ^{131}I-labeled anti-CD45 MAb, have been investigated (1-4). However, β-emitting radionuclides are not optimal for killing the targeted hematopoietic cells due to their long path length and low dose rates (5-8). Owing to the long β-particle path (i.e. mean range 0.4 to 5 mm (9)) the majority of the emitted energy is deposited outside of the targeted hematopoietic cells. Thus, while specific targeting of hematopoietic cells may be achieved with the MAb, the β-particles may deliver non-lethal doses to the targeted cells while causing non-specific toxicity to surrounding normal tissues.

In contrast to β-emissions, α-particles are characterized by very high linear energy transfer, with most of the particles' energy being deposited over only a few cell diameters (i.e. 40−90 μm). Given this favorable feature, we investigated bismuth-213 (^{213}Bi)-labeled anti-CD45 MAb as replacement for TBI in a nonmyeloablative conditioning regimen for HCT in a canine model (10-12). Although the treatment was effective in allowing successful engraftment of marrow, several pragmatic obstacles precluded translating ^{213}Bi-labeled MAbs into clinical studies including the very high cost of the parent radionuclide to ^{213}Bi, actinium-225 (^{225}Ac). Furthermore, adequate quantities of ^{225}Ac were not available for clinical studies.

Astatine-211 (^{211}At) is an alternative α-particle-emitting radionuclide for radioimmunotherapy (13). ^{211}At has a longer half-life than ^{213}Bi (7.21 h vs. 45.6 min), potentially making an ^{211}At-labeled anti-CD45 MAb more effective for targeting and killing hematopoietic cells. Based on our success with ^{213}Bi-labeled anti-CD45 MAb in conditioning for HCT, we compared biodistributions, myelosuppression and non-hematopoietic toxicities in mice with a MAb targeting hematopoietic tissues after radiolabeling it with either ^{211}At or ^{213}Bi. The antibody, a rat anti-murine CD45 MAb, 30F11 (2,14) used in the mouse provided a model for understanding differences between the two radionuclides.

Materials and Methods

Antibody and Chemicals

The rat anti-murine CD45 MAb, 30F11, is an IgG_{2b} MAb that recognizes all murine CD45 isoforms (2). The 30F11 hybridoma cell line was a gift from Dr. Irv Bernstein (Fred Hutchinson Cancer Research Center). The 30F11 MAb was produced by injecting the hybridoma into pristane-primed mice to generate ascites. The 30F11 MAb was purified from ascitic fluid by protein G immunoabsorption column chromatography. The protein-reactive ^{213}Bi-chelation reagent, isothiocyanatobenzyl-CHX-A″-DTPA (referred to as IB-CHX-A″) used to modify 30F11 was purchased from Macrocyclics (Dallas, TX). The ^{211}At-reactive protein modification reagent, N-(15-(aminoacyldecaborate)-4,7,10-trioxatridecanyl)-3-maleimidopropionamide (referred to as ADTM) was prepared as previously described (15).

Radionuclides

^{213}Bi was obtained by elution from an ^{225}Ac generator purchased from the US Department of Energy (Oak Ridge, TN) as previously described (10). ^{211}At was obtained by irradiating bismuth metal with a 28 MeV α-beam in a Scandatronix MC50 cyclotron housed in the Department of Radiation Oncology at the University of Washington. The ^{211}At was removed

from irradiated bismuth targets by dry distillation and isolated in 0.05 N NaOH as previously described (16).

Modification of MAb 30F11 for Radiolabeling

Modification of 30F11 for labeling with ^{213}Bi was achieved by conjugation of IB-CHX-A″ with 30F11 in 50 mM HEPES buffer, pH 8.5, at room temperature for 18 h as previously described (10). Rigorous demetallation was conducted before conjugation with the MAb and again after the MAb-conjugate was purified. Modification of 30F11 for labeling with ^{211}At was achieved by treatment with 10 mM dithiothreitol (DTT) for 1 hour at room temperature, followed by buffer exchange into 20 mM sodium phosphate at pH 6.5, containing 1 mM EDTA, then addition of 10 equivalents of ADTM in DMSO to the DTT-treated 30F11 with ADTM. After the conjugation reaction proceeded for 1 hour at room temperature, the reaction mixture was eluted on a PD-10 column (Sephadex G-25) pre-equilibrated in PBS, pH 7.2. The fractions containing protein were pooled and concentrated in a Centricon-30 to provide the 30F11-ADTM. The 30F11-ADTM conjugates were analyzed by size-exclusion HPLC and IEF to assess modification to the MAb.

Radiolabeling Methods

The 30F11-CHX-A″ conjugate was radiolabeled with ^{213}Bi in 0.3 M NH$_4$OAc, pH 4.2−4.5, for 2−5 min as described (10). The 30F11-ADTM conjugate was labeled with ^{211}At as follows. To 100−200 μL of 1−5 mg/mL solution of 30F11-ADTM conjugate in PBS was added 2−100 μL of Na[^{211}At]At, then 20−40 μL of chloramine-T (1 mg/mL) in H$_2$O. The reaction was allowed to proceed for 30 s to 2 min; then, 20−40 μL of a 1 mg/mL solution of Na$_2$S$_2$O$_5$ in H$_2$O was added to quench the reaction. The reaction mixture was then passed over a G-25 Sephadex column (PD-10, Pharmacia) eluting with 0.9% saline (15). Fractions were collected and those containing protein were combined. Radiochemical yield was determined by the amount of radioactivity associated with the protein relative to the amount of radioactivity placed on the column. Radiochemical purity of the radiolabeled proteins was determined by SE-HPLC.

Animal Studies

All mouse experiments were conducted under a protocol approved by the Fred Hutchison Cancer Research Center Institutional Animal Care and Use Committee. Female BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). All reagents were administered to the BALB/c mice in a total volume of approximately 200 μL via the lateral tail vein. Sets of 30 mice were injected with either ^{211}At- or ^{213}Bi-labeled MAb. Of those mice, 20 were sacrificed at predetermined times to obtain tissue distribution data, and the remaining 10 were evaluated over 8 weeks for myelosuppression and toxicities. All mice were weighed weekly to assess for toxicity.

A total of 13 biodistribution studies were conducted. Seven experiments were conducted with [^{211}At]30F11-ADTM (^{211}At-MAb) and six with [^{213}Bi]30F11-CHX-A″ (^{213}Bi-MAb). In the experiments, tissue distributions of conjugates containing varying quantities of both radioactivity and MAb were evaluated (i.e. 2 μCi/10 μg, 10 μCi/10 μg, 50 μCi/10 μg, 6 μCi/ 2 μg, 20 μCi/2 μg, 20 μCi/10 μg or 20 μCi/40 μg for labeled MAbs, and an additional experiment that had 20 μCi/10 μg for the ^{211}At-MAb). In ^{213}Bi (t$_{1/2}$ = 45.6 min) experiments, groups of 5 mice were sacrificed at 15, 45, 90 and 180 min after injection, when 20%, 50%, 75%, and 94% of the radionuclide had decayed, whereas for the ^{211}At (t$_{1/2}$ = 7.21 h) experimental groups mice were sacrificed 1, 3, 7 and 24 h after injection, when 9%, 25%, 49% and 90% of the radionuclide had decayed.

For evaluation of tissue concentrations of radioactivity, eight tissues were examined, including muscle, lung, kidney, spleen, liver, intestine, neck and stomach. The spleen was used as a surrogate tissue for hematopoiesis as total organ weight could be serially followed in addition to tissue concentrations of radioactivity. Bone marrows were sent for pathological examination only. Blood samples were obtained by heart puncture immediately after sacrificing the mice. Excised tissues were blotted free of blood and weighed. The total blood volume was estimated to be 6% of body weight (17). The radioactivity in each tissue was measured with a γ-counter (PACKARD® COBRA™ GMI, Inc., Minnesota, USA), and counts per minute were corrected for decay of each sample from the initiation of counting. Tissue concentrations of radioactivity were expressed as percentages of the injected dose per gram (%ID/g). The calculation of %ID/g was based on standards containing 1μL of the injected dose.

For pathological examination, selected tissues (spleen, liver, and bone marrow) were analyzed in untreated mice and those sacrificed at 24 h, 48 h, 1 week, 2 weeks and 1 month after injection of 10 μCi ^{211}At on 10 μg of MAb. Necropsies were also performed to investigate the causes of death in five mice given 50 μCi ^{211}At on 10 μg MAb (n=4) or 20 μCi ^{211}At on 40 μg of MAb (n=1) in which lethal toxicity occurred. Each tissue was fixed in 10% neutral buffered formaldehyde and then embedded in paraffin. Tissue sections were cut (4 μm) and stained with haematoxylin and eosin (HE staining) by an automatic staining system (Tissue-Tek® DRS™ 2000, Sakura Finetek U.S.A., Inc., CA).

Tissue Radiation Dose Estimates

Radiation absorbed doses were calculated for ^{213}Bi and ^{211}At in mouse tissues using standard methods for α-particle and beta (electron) dosimetry. Using the mathematical formalism established by the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine (18), the absorbed doses (cGy) to target tissues for α-particles and electrons or β-particles were calculated from the available nuclear decay data (19) and biodistribution data (see Supplemental Information). The time-integrated activity (or total number of disintegrations) in each tissue was determined for each radionuclide by plotting the activity-time curve, identifying an appropriate function to represent the plotted data (by least-squares linear regression analysis), and by integrating the best-fit regression curve from time 0 to infinity. The radiation absorbed doses were then calculated using the MIRD formalism. The total absorbed dose to each tissue was calculated as the sum of the alpha plus electron contributions.

Myelosuppression and Toxicities

Myelosuppression and non-hematological toxicities were evaluated in 10 surviving mice from each experimental group remaining after biodistribution studies. After injection of the labeled MAb, blood samples were obtained by retro-orbital bleeding at 3 hr for ^{213}Bi or 24 hr for ^{211}At, and weekly (alternating between two groups of 5 mice each) for a total of 8 weeks. At each time point, the blood from the 5 mice within the group was pooled and peripheral blood counts, liver enzymes, and kidney function were monitored in collected blood. Blood was also obtained from 5 control mice for peripheral blood counts or for chemistry and pooled to allow sufficient blood volume. Five percent ethylenediaminetetraacetic acid (EDTA) was used as anticoagulant for peripheral blood samples. White blood cell (WBC) counts, hemoglobin (Hb) level and platelet (Plt) counts were automatically measured by a quantitative automated hematology analyzer (the Sysmex XT2000i, Sysmex America, Mundelein, IL, USA). The analyses were conducted at the Seattle Cancer Care Alliance hematology laboratory. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (Bil), blood urea nitrogen (BUN) and creatinine (Cr) were measured to evaluate liver and renal toxicities. Additionally, to determine normal ranges [mean ± 2 standard deviations (SD)] of WBC counts, Hb level and Plt counts in peripheral blood, and AST, ALT, total Bil, BUN and Cr, as a baseline

for comparison, blood counts and chemistry data were also analyzed from 42 individual untreated female BALB/c mice. Blood chemistry analyses were conducted by the Department of Laboratory Medicine Research Testing Services at the University of Washington.

Results

Tissue Distributions

Tissue distributions were obtained for three different quantities (2, 10 and 40 μg) of ^{211}At-MAb or ^{213}Bi-MAb to determine which provided the most favorable biodistribution for therapy (i.e. had highest concentrations in spleen). Tissue concentrations were analyzed at four time points after injection to determine tissue radiation dose estimates. Concentrations (%ID/g) of radioactivity in selected tissues at the chosen time points after injection are shown in Figure 1. The spleen, which contains large numbers of CD45-containing hematopoietic cells, had the highest concentration of radioactivity in all experiments. Spleen concentrations were higher in the ^{211}At studies than in the ^{213}Bi studies, with the % injected dose/gram (mean ± standard deviation; %ID/g) ranging from 167±23 (1 hr) to 417±109 (24 hr) after injection of the ^{211}At conjugate and ranging from 45±9 (15 min) to 166±11 (3 h) after injection of the ^{213}Bi conjugate. Interestingly, spleen weights obtained at euthanasia at each time point decreased dramatically from 73.9 ± 13.0 to 33.1 ± 3.2 mg at 24 h after injection of ^{211}At (P<.0001, unpaired t test), but no weight changes in spleens were noted after injection of ^{213}Bi (data not shown). The spleen weight changes were reversible as mice euthanized at later time points had normal spleens weight 2 weeks after injection of ^{211}At. The liver contained the second highest concentrations, with study group averages ranging from 18−50 %ID/g for ^{211}At and 19−33 % ID/g for ^{213}Bi. The weights of the livers decreased slightly from 1.10 ± 0.09 to 0.80 ± 0.12 g at 1 h after injection in the ^{211}At studies, but the changes were reversible. Kidney concentrations were low, with group averages ranging from 8−10 %ID/g after ^{211}At and 7−8 %ID/g after ^{213}Bi. Blood concentrations were similar between ^{213}Bi and ^{211}At groups, and reflected the quantity of MAb injected. Of note, free ^{211}At would be expected to accumulate in the thyroid (as measured in the neck which contains the thyroid), lung and stomach.

Tissue Radiation Dose Estimates

The biodistribution data were used to estimate the tissue radiation doses when 2, 10 or 40 μg of ^{211}At-MAb or ^{213}Bi-MAb were administered (Table 1A). For comparison, the absorbed doses of ^{213}Bi were multiplied by 9.49 (difference in half-lives) to equate the total number of ^{213}Bi atoms to that of ^{211}At. As a further comparison, in Table 1B, the tissue doses obtained using 50 μCi or, hypothetically, 500 μCi ^{213}Bi (administered on the three different quantities of MAb) were compared to those obtained if 50 μCi of ^{211}At were administered. The 500 μCi ^{213}Bi value was an arbitrary value chosen as it would provide about the same radioactive dose as 50 μCi of ^{211}At taking into account the 9.49× half-life factor between the two isotopes.

Myelosuppression

Normal ranges determined for the WBC count, Hb, and Plt count in an untreated cohort of mice were 4.8 ± 3.4 (× 10^3 mm^3), 16.0 ± 2.0 (g/dl) and 92.3 ± 28.4 (× 10^4 mm^3), respectively. No significant cytopenias were observed in the ^{213}Bi groups (Figure 2). On the other hand, lethal myelosuppression was observed in all 5 mice receiving 20 μCi ^{211}At on 40 μg of MAb (data not shown in the figure) or 50 μCi ^{211}At on 10 μg MAb (minimal WBC count 0.21 × 10^3 and 0.12 × 10^3/mm^3, Plt counts 1.2 × 10^4 and 0.3 × 10^4/mm^3, Hb level 1.5 and 4.2 g/dl, respectively). Pancytopenias started to appear 1 week after injection and were irreversible. In the mice receiving 20 μCi ^{211}At on 10 μg of MAb, significant pancytopenias were observed, with nadirs at 2 weeks after injection (minimal WBC count 0.45 × 10^3/mm^3). However, the pancytopenias resolved at 3 weeks after injection. In mice receiving ^{211}At-MAb, leukopenia

appeared at 24 h after injection, except in mice receiving the lowest quantity (2 μg) of MAb. In contrast, leukopenia was not observed in mice administered [213]Bi-MAb.

Hepatic toxicity

The normal ranges [mean ± 2 SD] for AST, ALT, and total Bil were 129 ± 96 (U/L), 52 ± 31 (U/L) and 0.48 ± 0.27 (mg/dl), respectively, in an untreated cohort of mice. Severe but non-lethal hepatic toxicity was observed at 3 hr after injection of 50 μCi [213]Bi on 10 μg of MAb (maximal AST 1329 U /L and ALT 928 U /L) (Figure 3A). This hepatic toxicity resolved by day 15. In all [213]Bi groups, except for mice given 2 μCi [213]Bi on 2 μg of MAb, mild temporary hepatic enzyme elevations were detected at 3 h. However, the values recovered to near normal levels at 1 week. On the other hand, no significant hepatic toxicity was observed in any [211]At-treated group.

Renal toxicity

The normal ranges (mean ± 2 SD) determined for BUN and Cr were 21.5 ± 7.8 (mg/dl) and 0.35 ± 0.17 (mg/dl), respectively, in an untreated cohort of mice. No significant renal toxicity was observed in mice administered either [213]Bi or [211]At (Figure 3B).

Tissue pathology ([211]At)

Mice in all experimental study groups gained weight over the study period, except the group receiving 20 μCi [211]At on 40 μg of MAb. In the group receiving 10 μCi [211]At, the bone marrow demonstrated progressive hypocellularity, and hematopoiesis significantly decreased at 24 h and 48 h after injection (Figure 4A). However, hematopoietic recovery occurred by 1 week after injection. Similarly, red pulp in the spleen significantly decreased and white pulp in the spleen became atrophic at 24 h and 48 h after injection (Figure 4B). In the 24 h sample, there was diffuse necrosis of lymphoid cells intermixed with proliferating lymphocytes. The white pulp became atrophic (approximately 25% of normal cellularity) and red pulp was depleted down to about 50% of normal cellularity at 24 h. Megakaryocytes remained. However, recovery of hematopoiesis in the spleen was also observed in the specimen 1 week after injection. There were no pathological abnormalities in the liver. In the five animals on which necropsies were performed, there were no abnormalities noted in the liver or kidney.

Necropsies were performed on mice that received 20 μCi [211]At on 40 μg of MAb or 50 μCi [211]At on 10 μg of MAb to investigate the cause of spontaneous death. Based on the autopsy and pathological examination, it was likely that all the mice in these two groups injected with [211]At labeled MAb died of complications of severe pancytopenia (such as anemia or sepsis). In contrast to the group receiving 10 μCi [211]At, the bone marrow cellularity remained very sparse at day 8 or 10 in groups receiving 20−50 μCi [211]At, documenting protracted myelosuppression (Figure 4C, row a). At day 8−11, the red and white pulps of the spleen were depleted down to 25% of normal cellularity and megakaryocytes almost disappeared in the groups receiving 20 μCi [211]At. In the groups receiving 50 μCi [211]At, the red and white pulps were depleted down to 5−10% of normal cellularity and megakaryocytes were rarely observed (Figure 4C, row b). There was no evidence of extramedullary hematopoiesis in the liver.

Discussion

The current study demonstrated that [211]At was more effective than [213]Bi at producing myelosuppression for the same quantity of injected radioactivity. All mice injected with 20 or 50 μCi [211]At but none with the same quantities of [213]Bi had lethal myeloablation. Severe reversible acute hepatic toxicity occurred with the highest doses of [213]Bi, but not with lower doses of [213]Bi or with any dose of [211]At. No renal toxicity occurred with either radionuclide. The data suggest that smaller μCi quantities of [211]At-labeled anti-CD45 antibody are sufficient

to achieve myelosuppression and myeloablation with less non-hematological toxicity compared with ^{213}Bi-labeled antibody.

Our previous study showed that the donor chimerism levels in dogs conditioned with ^{213}Bi-labeled anti-TCRαβ MAb (CA15.9D95) were lower than those observed in dogs conditioned with ^{213}Bi-labeled pan-hematopoietic anti-CD45 MAb (CA12.10C12) (10,11,20). The results suggested that ^{213}Bi-labeled anti-CD45 MAb was more effective in killing host residual cells, such as natural killer (NK) cells, that are responsible for graft rejection. CD45 was an excellent target because it is ubiquitously expressed on both nonmalignant and malignant hematopoietic cells (21-23). However, the short half-life of ^{213}Bi presents a significant problem since it mandates the use of large ^{225}Ac/^{213}Bi generators and multiple preparations/injections per patient to obtain adequate doses of clinical materials for patient treatment. A further consideration is the fact that, at present, there is a very high cost to obtain a generator of sufficient size to conduct clinical studies. Therefore, a clinical study was not feasible at the current time using ^{213}Bi. Additionally, the longer half-life of ^{211}At has logistical and, potentially, therapeutic benefits.

^{211}At is available at our institution by irradiation of a bismuth target with an α-particle beam from a cyclotron. An important consideration for initiating the investigation with ^{211}At was the fact that recent upgrades on the cyclotron and target station used to produce ^{211}At make it possible to prepare the quantities required for clinical studies, and this can be done at a much lower cost relative to producing ^{213}Bi. Perhaps more importantly, the consideration for studies where ^{213}Bi is replaced by ^{211}At is the fact that the half-life of ^{211}At ($t_{1/2} = 7.21$ h) is ~9.5× longer than that of ^{213}Bi ($t_{1/2} = 45.6$ min). This difference in half-life has some important benefits. One benefit is that there are 9.5 times the number of radioactive atoms as that of ^{213}Bi in each mCi of ^{211}At injected. Thus, for the same number of mCi of ^{211}At as ^{213}Bi, much higher doses can be obtained, or considerably lower quantities of ^{211}At might be used to deliver a therapeutic dose. Another benefit of the longer half-life is the fact that a smaller percentage of the injected radioactivity will decay during the period of targeting hematopoietic cells, potentially resulting in more specific delivery of the radiation.

In the current study, labeling MAb 30F11 with the two radionuclides required use of different chemical modifications that could potentially affect the tissue distribution. Further, biodistribution of the radiolabeled MAb conjugates (30F11-CHX-A″ and 30F11-ADTM) were only relevant over the period where most of the radioactivity decays, potentially making the relative biodistributions very different given the different half-lives of the two isotopes. To label ^{213}Bi, MAb 30F11 was conjugated with a benzylisothiocyanate-cyclohexyl derivative of diethylenetetraaminepentaacetic acid (IB-CHX-A″) which had been used in our previous canine conditioning studies. MAb conjugates of IB-CHX-A″ were rapidly labeled (5 min) in high yield (>80%) and provided good in vivo stability during the period of ^{213}Bi decay. Stability of the label had been a problem for antibodies labeled with ^{211}At (24). Although ^{211}At-labeled benzoate esters could be used for stable labeling some MAbs, an investigation of 30F11 labeled with N-hydroxysuccinimidyl 3-[^{211}At]astatobenzoate (25) indicated that it was not stable in vivo (unpublished). Therefore, an alternate ^{211}At-labeling conjugate, a recently developed reagent (15,26), N-(15-(aminoacyldecaborate)-4,7.10-trioxatridecanyl)-3-maleimide (ADTM), was used. The use of MAb-ADTM conjugates provided high radiochemical yields (70−80%) through direct labeling of ^{211}At, and provided high in vivo stability to deastatination.

The biodistribution studies showed that the much higher radiation doses delivered by ^{211}At depleted the hematopoietic cells and accounted for the differences seen in the spleen weights. While a large portion of the difference in radiation doses was provided by the fact that there are 9.49 times more ^{211}At atoms per μCi administered, it appears that another factor of 2× in

the dose may be due to the longer half-life of ^{211}At, permitting more localization of MAb to the spleen prior to decay. Thus, from the tissue radiation dose estimates, 500 μCi of ^{213}Bi would deliver less than half the dose to the target (spleen) of 50 μCi ^{211}At if the radionuclides were on 10 μg of MAb. As might be expected from the biodistribution and tissue dose estimates, the blood count data indicated that myelosuppression was more effective with the ^{211}At-MAb for the same μCi amount of ^{213}Bi-MAb.

In this study, severe hepatic toxicity appeared in the mice receiving the highest dose (50 μCi) of ^{213}Bi-MAb, presumably due to the abundance of hematopoietic cells and Kupffer cells in the liver expressing the CD45 antigen. There may also be a non-specific dose contribution as immunoglobulins from the blood stream are known to be trapped in the liver, resulting in the radiolabeled MAb being trapped even though CD45 is not expressed on hepatocytes (7). In the previous canine study, the dog receiving the highest dose of 8.8 mCi/kg ^{213}Bi labeled anti-CD45 MAb also showed marked elevation of hepatic enzymes and evidence of liver failure with the development of ascites due to toxicity from the radioimmunotherapy (10). Based on the present and previous data, hepatic toxicity of ^{213}Bi-labeled anti-CD45 MAb can be considered dose-limiting. The observed hepatic toxicity in the ^{213}Bi studies was likely caused by deposition of ^{213}Bi-labeled MAb in the liver. The nature of conjugate molecule (i.e. CHX-A″ or ADTM) to label the MAb with radionuclide might also contribute to hepatic deposition, but there are no data suggesting that either conjugate specifically localizes to liver.

The overall objective of our continuing research effort is to determine if MAbs labeled with an α-particle emitting radionuclide can deliver a marrow-ablative dose without the other organ toxicities associated with high-dose conditioning regimens. Our earlier studies demonstrated that ^{213}Bi-labeled anti-CD45 MAb provided adequate myelosuppression to obtain stable chimeras in the dog model. From previous dog data it was estimated that 1.5−2 mCi ^{213}Bi/kg on 0.5 mg anti-CD45 MAb/kg would likely be required in patients to obtain stable engraftment. This study demonstrated that ^{211}At was more effective at myelosuppression for the same quantity of radioactivity injected than ^{213}Bi without significant non-hematopoietic toxicity. Based on the fact that there are fewer barriers to clinical studies with ^{211}At, and the encouraging results obtained in this investigation, further studies in the dog model with ^{211}At-labeled anti-CD45 MAb are underway.

Acknowledgments

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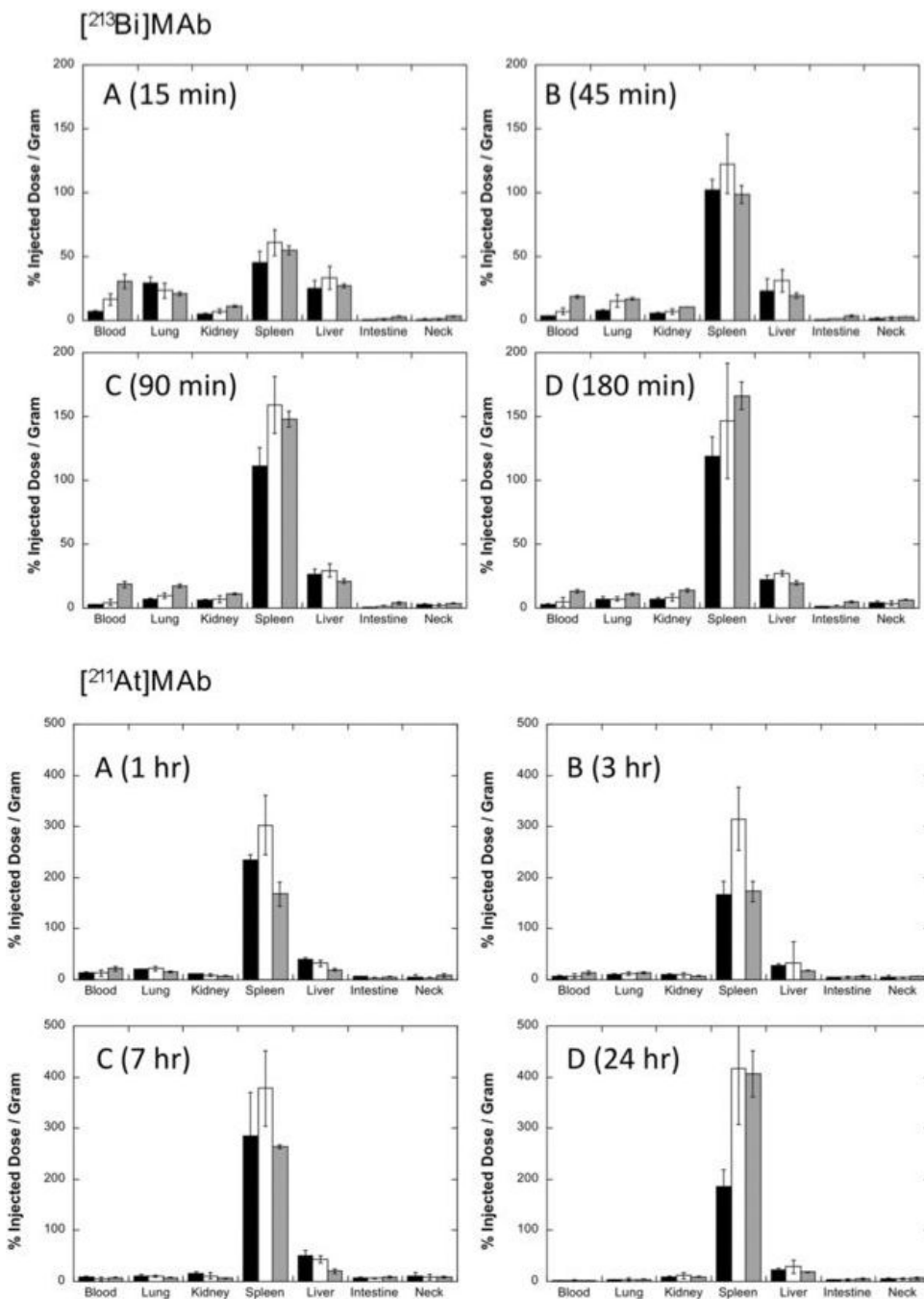
Figure 1. 213Bi- and 211At-labeled rat anti-mouse anti-CD45 antibody 30F11 conjugate (213Bi- and 211At-MAb) biodistributions

Tissue biodistributions were obtained in mice to assess MAb targeting to spleen, which has high concentrations of CD45-containing cells, and to determine the effect of varying the quantity of MAb on tissue concentrations. The graphs of the tissue concentrations, expressed as percent injected dose / gram (%ID/g), for studies that employed 2 μg (black bars), 10 μg (white bars) or 40 μg (gray bars) of MAb are shown. Data were obtained at 15, 45, 90 and 180 min after injection of 213Bi-MAb and at 1, 3, 7 and 24 h after injection of 211At-MAb. Data were obtained from groups of 5 mice per time point and were plotted as average values ± one standard deviation. Values plotted for injections of 2 and 40 μg quantities of MAb were

obtained from single experiments (5 mice per time point), whereas values plotted for 10 μg were averaged from 3 (^{213}Bi) or 4 (^{211}At) separate experiments (total of 15−20 mice per time point) because the biodistributions of MAb labeled with a specific radionuclide differed only when the quantities of MAb were different. Note that the y-axis maximum is 200 %ID/g for ^{213}Bi-MAb and 500 %ID/g for ^{211}At-MAb.
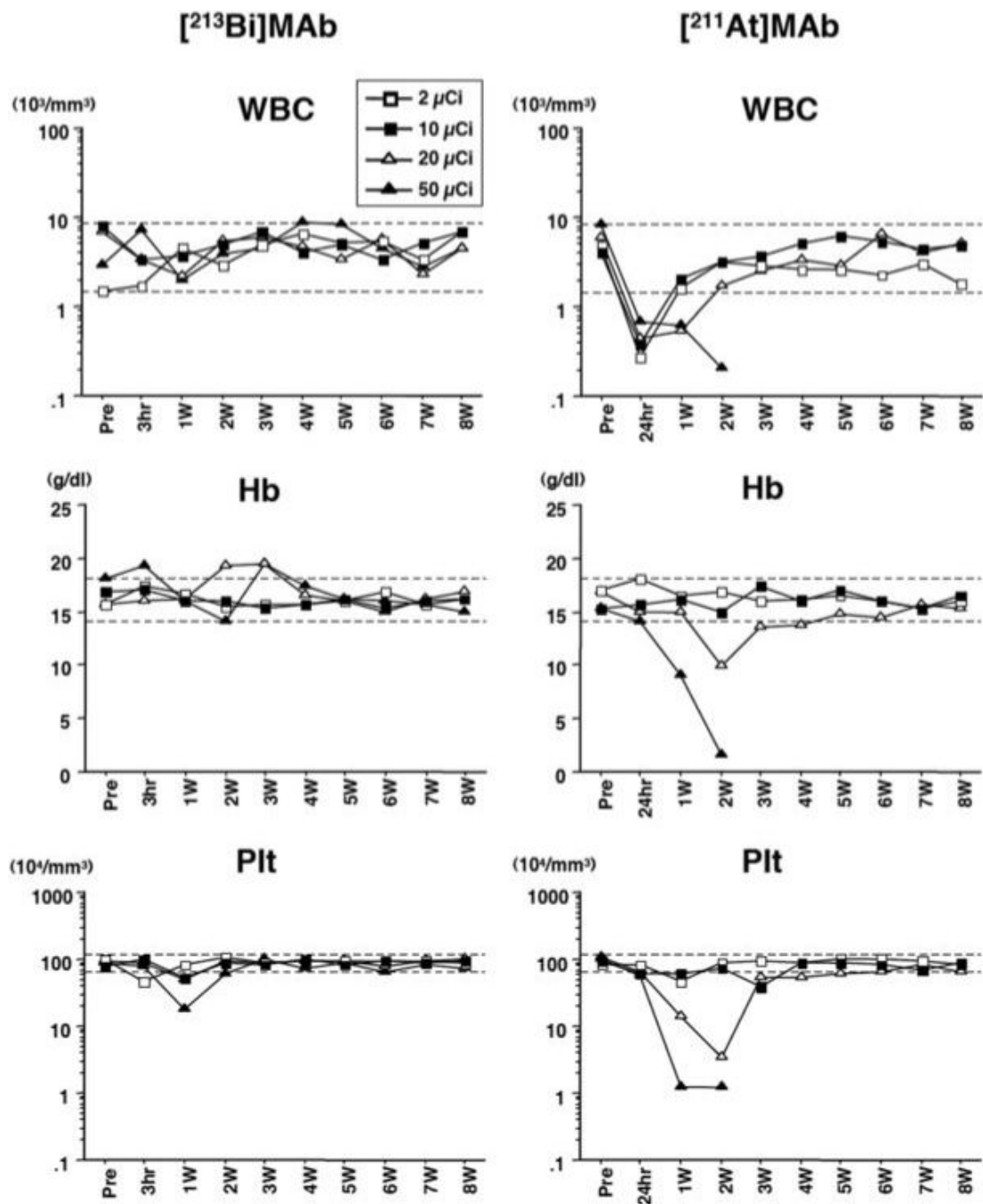
Figure 2. Myelosupression with varying amounts (2, 10, 20, 50 μCi) of ^{213}Bi-MAb or ^{211}At-MAb
Peripheral blood counts were monitored in collected blood at 180 min and then weekly
for ^{213}Bi and at 24 h and then weekly for ^{211}At. White blood cell (WBC) counts, hemoglobin
(Hb) levels and platelet (Plt) counts were monitored up to 8 weeks after injection. The displayed
data were obtained from mice treated with 2, 10, 20 and 50 μCi of ^{213}Bi or ^{211}At on 10 μg of
MAb. The dashed lines indicate normal ranges (mean ± 2 standard deviations) which were
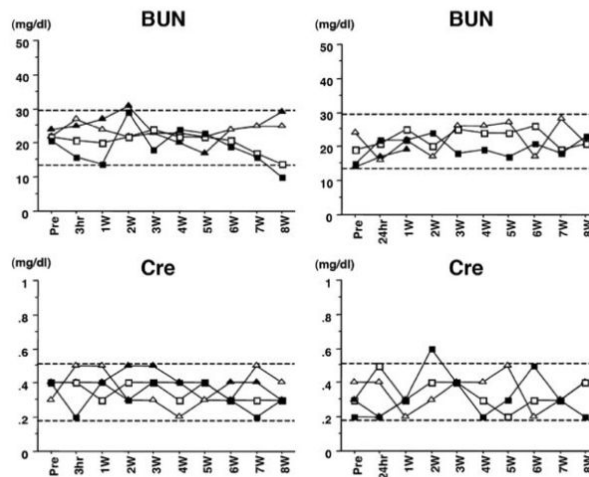calculated with data obtained from 42 untreated female BALB/c mice.
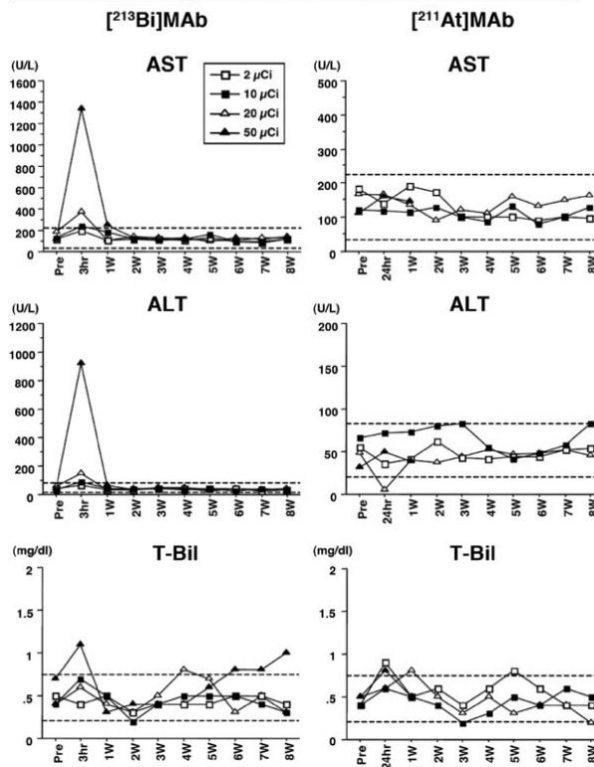
Figure 3. Hepatic and renal toxicity with varying amounts (2, 10, 20, 50 μCi) of ^{213}Bi-MAb ^{211}At-MAb

Hepatic and renal toxicities were monitored in sera from peripheral blood collected at 180 min (^{213}Bi) or 24 h (^{211}At) after injection, and then weekly. Liver toxicity was assessed by monitoring the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and total bilirubin (T-Bil). Renal toxicity was assessed by monitoring the blood urea nitrogen (BUN) and creatinine (Cre). The monitoring was performed up to 8 weeks after injection. The displayed data were obtained from mice treated with 2, 10, 20 and 50 μCi of ^{213}Bi or ^{211}At on 10 μg of MAb. The dashed lines indicate normal ranges (mean ± 2 standard deviations) which were calculated with data obtained from 42 untreated female BALB/c mice.
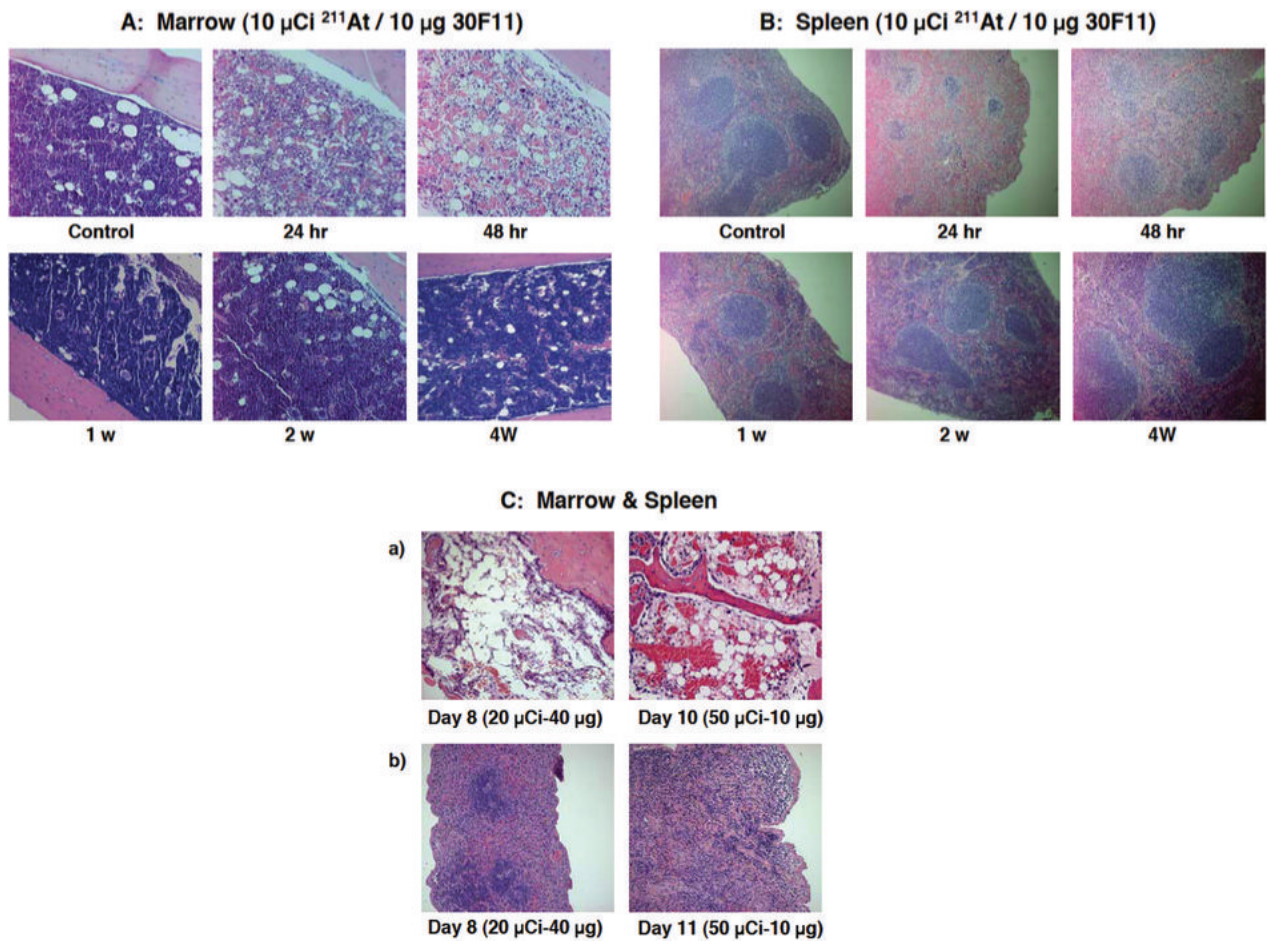
Figure 4. Pathological changes in the spleen and bone marrow after injection of [211]At-MAb
Pathological examination of bone marrow from femurs **(Panel A)** and the spleens **(Panel B)** was performed on mice sacrificed 24, 48 h, 1, 2, 4 weeks after injection of 10 µCi/10 µg [211]At-MAb. Pathological examination of the bone marrow from femur or sternum was conducted on necropsy samples on day 8 and day 10 after 20 µCi/40 µg or 50 µCi/10 µg [211]At-MAb were administered, respectively **(Panel C; row a)** and the spleens on day 8 and day 11 after 20 µCi/40 µg or 50 µCi/10 µg [211]At-MAb were administered, respectively **(Panel C; row b)**.

Table 1

Calculated tissue dose estimates for [^{213}Bi]30F11 and [^{211}At]30F11.

A. Radiation absorbed doses per unit administered activity (cGy / µCi)

Nuclide	Bi-213	Bi × 9.49*	At-211	Bi-213	Bi × 9.49*	At-211	Bi-213	Bi × 9.49*	At-211
MAb wt	2 µg	2 µg	2 µg	10 µg	10 µg	10 µg	40 µg	40 µg	40 µg
Blood	0.78	7.38	9.00	1.28	12.15	8.01	3.42	32.46	12.60
Muscle	0.19	1.84	1.11	0.19	1.84	1.96	0.31	2.95	1.98
Lung	2.68	25.43	13.30	2.35	22.30	14.67	2.82	26.76	12.30
Kidney	1.13	10.72	15.70	1.42	13.48	17.30	2.24	21.26	12.10
Spleen	17.80	168.92	327.00	23.30	221.12	589.00	21.40	203.09	419.00
Liver	4.12	39.10	52.10	5.13	48.68	47.80	3.71	35.21	27.00
Intestine	0.16	1.47	7.02	0.27	2.58	7.32	0.74	7.01	9.13
Neck	0.37	3.50	11.10	0.43	4.06	8.82	0.72	6.82	10.60
Stomach	0.19	1.84	3.35	0.21	2.03	7.77	0.62	5.90	4.49

B. Organ or tissue absorbed dose (cGy) through complete decay of the activities administered.

Nuclide	Bi-213	Bi-213	At-211	Bi-213	Bi-213	At-211	Bi-213	Bi-213	At-211
MAb wt	2 µg	2 µg	2 µg	10 µg	10 µg	10 µg	40 µg	40 µg	40 µg
Activity	50 µCi	500 µCi**	50 µCi	50 µCi	500 µCi**	50 µCi	50 µCi	500 µCi**	50 µCi
Blood	39	389	450	64	640	401	171	1710	630
Muscle	10	97	56	10	97	98	16	156	99
Lung	134	1340	665	118	1175	734	141	1410	615
Kidney	57	565	785	71	710	865	112	1120	605
Spleen	890	8900	16350	1165	11650	29450	1070	10700	20950
Liver	206	2060	2605	257	2565	2390	186	1855	1350
Intestine	8	78	351	14	136	366	37	370	457
Neck	18	185	555	21	214	441	36	360	530
Stomach	10	97	168	11	107	389	31	311	225

* This value represents an equivalent number of ^{213}Bi atoms to ^{211}At atoms obtained by multiplying by a half-life difference factor (9.49×)

** The 500 µCi is an arbitrary value chosen to provide about the same dose to most tissues; 10× is similar to 9.49× half-life factor.