Efforts to Control the Errant Products of a Targeted *In vivo* Generator

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Abstract

Alpha-particle immunotherapy by targeted α-emitters or α-emitting isotope generators is a novel form of extraordinarily potent cancer therapy. A major impediment to the clinical use of targeted actinium-225 (^{225}Ac) *in vivo* generators may be the radiotoxicity of the systemically released daughter radionuclides. The daughters, especially bismuth-213 (^{213}Bi), tend to accumulate in the kidneys. We tested the efficacy of various pharmacologic agents and the effect of tumor burden in altering the pharmacokinetics of the ^{225}Ac daughters to modify their renal uptake. Pharmacologic treatments in animals were started before i.v. administration of the HuM195-^{225}Ac generator. ^{225}Ac, francium-221 (^{221}Fr), and ^{213}Bi biodistributions were calculated in each animal at different time points after ^{225}Ac generator injection. Oral metal chelation with 2,3-dimercapto-1-propanesulfonic acid (DMPS) or meso-2,3-dimercaptosuccinic acid (DMSA) caused a significant reduction ($P < 0.0001$) in the renal ^{213}Bi uptake; however, DMPS was more effective than DMSA ($P < 0.001$). The results with DMPS were also confirmed in a monkey model. The renal ^{213}Bi and ^{221}Fr activities were significantly reduced by furosemide and chlorothiazide treatment ($P < 0.0001$). The effect on renal ^{213}Bi activity was further enhanced by the combination of DMPS with either chlorothiazide or furosemide ($P < 0.0001$). Competitive antagonism by bismuth subnitrate moderately reduced the renal uptake of ^{213}Bi. The presence of a higher target-tumor burden significantly prevented the renal ^{213}Bi accumulation ($P = 0.003$), which was further reduced by DMPS treatment ($P < 0.0001$). Metal chelation, diuresis with furosemide or chlorothiazide, and competitive metal blockade may be used as adjuvant therapies to modify the renal accumulation of ^{225}Ac daughters. (Cancer Res 2005; 65(11): 4888-95)

Introduction

Radioimmunotherapy with α-particle–emitting radionuclides may have advantages because α-particles have high energies and short path lengths (50-80 μm; refs. 1, 2). Therefore, a large amount of energy is deposited over a short distance, which renders α-particles extremely cytotoxic with a high relative biological effectiveness (3–5). Little collateral damage to surrounding normal, antigen-negative cells occurs (4, 6). A single traversal of densely ionizing, high-energy α-particle radiation through the nucleus may be sufficient to kill a target cell (7). In addition, double-stranded DNA damage caused by α-particles is not easily repaired by the cells and this cytotoxicity is largely unaffected by the oxygen status and cell cycle position of the cells (1).

Among the α-emitters, we have chosen actinium-225 (^{225}Ac), the α-emitting atomic generator, as a possible candidate for use in radioimmunotherapy. ^{225}Ac has a sufficiently long half-life (10 days) for feasible use and it decays to stable bismuth-209 via six atoms, yielding a net of four α-particles (Fig. 1; ref. 8). We have been able to successfully couple ^{225}Ac to internalizing monoclonal antibodies using 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as the chelating moiety. The ^{225}Ac-DOTA antibody construct acts as a tumor-selective, molecular-sized, *in vivo* atomic generator (targetable *in vivo* generator) of α-particle–emitting elements (8). The ^{225}Ac-DOTA antibody constructs have been shown to be safe and potent antitumor agents in mouse models of solid prostatic carcinoma, disseminated lymphoma, and i.p. ovarian cancer (9, 8). The safety of ^{225}Ac-HuM195 and ^{225}Ac-3F8 both at low doses has been shown in primates (10). Therefore, these agents possess the potential to be used clinically in the future to treat a variety of tumor types.

^{225}Ac decays via its α-emitting daughters, francium-221 (^{221}Fr), astatine-217 (^{217}At), and bismuth-213 (^{213}Bi), to stable nonradioactive ^{209}Bi (8, 11). These daughters, once formed, are unlikely to associate with the antibody-DOTA construct due to high atomic recoil-energy as a result of α-decay (12), possible rupture of the chelate, and different chemical properties of the daughters. The daughters generated and retained inside the cancer cell after internalization of the ^{225}Ac-labeled antibody add to its cytotoxic effect (8). We have shown tumor burden as an important determinant in the biodistribution of the antibody (13, 14). However, the free daughters produced in the vasculature from the circulating unbound antibody or the antibody bound to the surface of a target cell could diffuse or be transported to various target organs, where they can accumulate and cause radiotoxicity (11). Bismuth is known to accumulate in the renal cortex (15–17). Although safe at low doses, monkeys injected with escalating doses of a nonspecific ^{225}Ac *in vivo* generator developed a delayed radiation nephropathy, manifesting as anemia and renal failure (10). Therefore, a possible hindrance to the development of these agents as safe and effective cancer therapeutics is likely to be their nephrotoxicity. By preventing the renal accumulation of the radioactive daughters or by accelerating their clearance from the body, the therapeutic index of the ^{225}Ac *in vivo* generator could be enhanced.

^{217}At has the shortest half-life (32 milliseconds) of the α-emitting daughters of ^{225}Ac. It decays almost instantaneously to ^{213}Bi. ^{213}Bi and ^{221}Fr have relatively longer half-lives (45.6 and 4.9 minutes, respectively; ref. 11) and, therefore, have the potential to cause radiation damage. The presence of bismuth-binding, metallothionein-like proteins in the cytoplasm of renal proximal tubular cells makes the kidney a prime target for the accumulation

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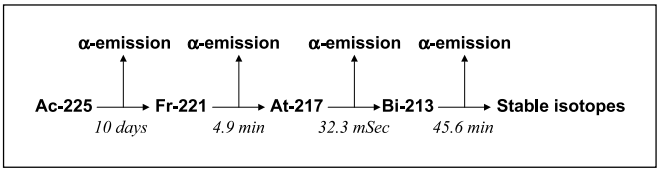

Figure 1. Simplified ^{225}Ac generator to ^{213}Bi decay scheme, yielding four net α-particles. The half-lives are shown in italics.

of free, radioactive bismuth (15–17). Dithiol chelators have been shown to chelate bismuth and enhance its excretion in various animal, as well as human, studies (12, 18–20). ^{221}Fr is another potentially toxic daughter of ^{225}Ac. Francium, like sodium and potassium, is an alkali metal. Furosemide and thiazide diuretics are known to accelerate the elimination of sodium and potassium in urine by inhibiting their reabsorption in different segments of the nephron (21).

In this study, we show the effectiveness of metal chelation in scavenging and, therefore, preventing the renal accumulation of free, radioactive bismuth. We also show the value of pharmacologic inhibition of metal reabsorption in different segments of the nephron in accelerating the clearance of the radioactive ^{225}Ac daughters. Lastly, we report the effectiveness of competitive metal blockade and the role of tumor burden in altering the pharmacokinetics and uptake of the daughter radionuclides in the kidneys. Each of these interventions could reduce the renal accumulation of ^{225}Ac daughters.

Materials and Methods

Animals. Female BALB/c and severe combined immunodeficient (SCID) mice, 4 to 12 weeks of age, were obtained from Taconic (Germantown, NY). All animal studies were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Memorial Sloan Kettering Cancer Center.

Preparation and quality control of ^{225}Ac-labeled antibodies. ^{225}Ac (Oak Ridge National Laboratory, Oak Ridge, TN) was conjugated to SJ25C1 (mouse antihuman CD19 IgG1; Monoclonal Antibody Core Facility, Memorial Sloan Kettering Cancer Center) or HuM195 (humanized anti-CD33 IgG1; Protein Design Labs, Fremont, CA) using a two-step labeling method as described previously (22). Routine quality control of the labeled antibody was done using instant TLC to estimate the radiopurity and cell-binding assay to determine the immunoreactivity (9, 23).

Administration of ^{225}Ac *in vivo* generator to mice. The mice were anesthetized and then injected i.v. (in retro-orbital venous plexus) with 0.5 μCi ^{225}Ac-labeled HuM195 (for chelation, diuresis, and competitive metal blockade experiments). For the tumor burden experiments, we used ^{225}Ac-labeled SJ25C1. The injected volume was 100 μL and the antibody dose was 6 to 8 μg per animal.

Free-metal scavenging with 2,3-dimercapto-1-propanesulfonic acid, meso-2,3-dimercaptosuccinic acid, or calcium-diethylenetriamine pentaacetate. Animals received either 2,3-dimercapto-1-propanesulfonic acid (DMPS; Sigma, St. Louis, MO) or meso-2,3-dimercaptosuccinic acid (DMSA; Sigma) in drinking water (1.2 and 1.5 mg/mL, respectively) starting 1 day before injection with ^{225}Ac generator and continued until the animals were sacrificed. The control animals received regular drinking water. The water bottles were replaced with fresh DMPS or regular water every 12 hours. The average water consumption was slightly lower in the DMPS-treated group (3.5 mL/mouse/d) compared with control animals (4 mL/mouse/d). For i.p. chelation therapy, 1.2 mg calcium-diethylenetriamine pentaacetate (Ca-DTPA) or DMPS was administered per animal in 2-hourly divided doses over 24 hours. Animals (five per group) were sacrificed at different time points postinjection by carbon dioxide asphyxiation, and samples of blood (by cardiac puncture) and kidneys were removed. The organs were washed in distilled water, blotted dry on gauze, weighed, and the activities of ^{221}Fr (185-250 keV window) and ^{213}Bi (360-480 keV window) were measured using a gamma counter (COBRA II, Packard Instrument Company, Meriden, CT). Samples of the injectate (100 μL) were used as decay correction standards. Adjustment was made for the small percentage of bismuth activity that counted in the francium activity window. Percentage injected dose of ^{225}Ac, ^{221}Fr, and ^{213}Bi per gram of tissue weight (%ID/g) was calculated for each animal at the time of sacrifice, using the equation described previously (24). The mean %ID/g was determined for each experimental group.

Scavenging of free metal in monkeys. Male cynomolgus monkeys (n = 2), weighing 7 to 8 kg, were injected i.v. with 22.5 μCi ^{225}Ac-labeled antibody (HuM 195). The treated animal received DMPS in drinking water (1.2 mg/mL) and was injected i.v. with 200 mg DMPS (Heyl, Berlin, Germany) 90 minutes before sacrifice. The control animal received regular drinking water. The monkeys were sacrificed 24 hours postinjection with ^{225}Ac-labeled antibody. Percentage injected dose of ^{225}Ac, ^{221}Fr, and ^{213}Bi per gram of blood and kidneys (%ID/g) at the time of sacrifice was calculated for both monkeys as described above.

Intra-peritoneal hydration in mice. The mice were randomized to receive either 1 mL normal saline i.p. every 12 hours or no treatment (control group). Mice (four animals per group) were sacrificed at 24 and 192 hours after ^{225}Ac *in vivo* generator injection. The mean activity of actinium, francium, and bismuth per gram (%ID/g) in blood and kidneys was determined for hydration and control group. To evaluate the effect of hydration on blood clearance of the parent drug ([^{225}Ac]HuM195), mice received either hydration (1 mL normal saline i.p. every 12 hours) or no hydration. Four to six mice from each group were bled from the retro-orbital venous plexus at 1, 4, 24, 120, and 192 hours after injection with the Ac-labeled antibody. The percentage injected dose of actinium per gram of blood was calculated for each animal. The mean %ID/g in blood was determined for the hydration treatment and control group and plotted against time.

Forced diuresis in mice. In one study, mice were randomized to furosemide treatment, chlorthiazide treatment, or no treatment (control) groups. Furosemide and chlorothiazide were administered i.p. The loading doses of furosemide and chlorothiazide were 250 and 750 mg/kg, respectively, administered 1 hour before ^{225}Ac *in vivo* generator injection. The maintenance doses were 100 and 300 mg/kg, respectively, administered 12 and 24 hours after the loading dose. The controls were injected with an equal volume of saline (vehicle). In another study, mice received DMPS (1.2 mg/mL in drinking water) and either furosemide or chlorothiazide i.p. (same dose schedule as above). The controls received regular drinking water and were injected with equal volume of saline. To study the effect of a potassium-sparing diuretic on daughter excretion, mice received either a s.c. sustained time release (1.1 mg/d) spironolactone pellet (Innovative Research of America, Sarasota, FL), implanted 12 hours preinjection with ^{225}Ac *in vivo* generator, or no treatment (controls). Animals (five per group) were sacrificed at 24 hours postinjection with the labeled antibody and the mean activity (%ID/g) of ^{225}Ac, ^{221}Fr, and ^{213}Bi in blood and kidneys was calculated for each experimental group.

Competitive metal blockade studies. Mice were injected i.p. with 200 μL 1% bismuth subnitrate suspension (100 mg/kg; Sigma) 4 hours before ^{225}Ac *in vivo* generator injection (n = 5; sacrificed at 6 hours postinjection with ^{225}Ac *in vivo* generator) or 4 hours before and 8 and 20 hours after ^{225}Ac *in vivo* generator injection (n = 5; sacrificed at 24 hours postinjection). Controls (n = 10) received an equal volume of saline (vehicle) i.p. and five animals were sacrificed at 6 and 24 hours postinjection. In another experiment, mice received either 200 μL of 10 mg/mL ferric ammonium citrate solution (Sigma) or saline (vehicle) i.p. 1 hour before ^{225}Ac *in vivo* generator injection. The animals were sacrificed 24 hours postinjection. The mean activity (%ID/g) of actinium, francium, and bismuth in blood and kidneys at sacrifice time was estimated for each experimental group.

Tumor burden experiments. Disseminated human Daudi lymphoma (25) treated with ^{225}Ac-labeled anti-CD19 was used as the model system. SCID mice, 10 to 12 weeks old, were randomized to "low tumor burden" group (7 days growth of tumor), "high tumor burden" group (30 days growth of tumor), or "high tumor burden + DMPS" group (30 days growth of tumor and treated with 1.2 mg/mL DMPS in drinking water starting 1 day before injection with ^{225}Ac *in vivo* generator). All mice were injected i.v. with 5×10^6 Daudi lymphoma cells in 0.1 mL PBS. The "low-burden" animals were injected with the tumor cells 23 days after the "high-burden" ones. The animals were checked daily for the onset of hind leg paralysis. Thirty days after injection of tumor cells in the high-burden animals (7 days for the low-burden group), all animals were injected retro-orbitally with 0.5 μCi ^{225}Ac-labeled SJ25C1 in 100 μL. The animals (five per group) were sacrificed at 24 hours postinjection, and the mean ^{225}Ac, ^{221}Fr, and ^{213}Bi activity (%ID/g) in blood, femurs, and kidneys was calculated for each experimental group. The percentage of human CD20–positive cells in the femoral bone marrow was estimated in one representative animal from the high- and low-burden groups by flow cytometric staining with phycoerythrin-conjugated antihuman CD20 (BD Biosciences, San Jose, CA) and compared with that of a nontumor-bearing mouse of the same strain.

Estimation of renal absorbed dose. The renal ^{225}Ac, ^{221}Fr, and ^{213}Bi activity (%ID/g) at various time points (to 8 days) postinjection with ^{225}Ac-labeled HuM195 was used to estimate the renal absorbed dose. The mean absorbed dose to the kidneys was estimated by assuming that all α-particle energy emitted by decays within the kidneys is absorbed within the kidneys. A biexponential expression, $A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$, was fitted to the renal time versus ^{225}Ac activity curve using the Simulation Analysis and Modeling software package, SAAM II (SAAM Institute, Inc., Seattle, WA; ref. 10). The resulting expression was analytically integrated to 8 days (192 hours) and to infinity to obtain the number of ^{225}Ac decays in the kidneys over 8 days and the total number of decays, respectively. Biexponential expressions were also fitted to the kidney time-activity curves for ^{221}Fr and ^{213}Bi. The resulting expressions were integrated over the measurement period of 8 days. The total number of decays of these two daughters was obtained by scaling the estimated decays for each daughter during the data collection period by the ratio of total ^{225}Ac decays to ^{225}Ac decays estimated during the 8-day measurement period. This approach assumes that the daughter radionuclides produced by ^{225}Ac decay will continue to concentrate in the kidneys at the ratio observed over the first 8 days. Because of its short (32 milliseconds) half-life and the correspondingly low probability of its leaving the kidneys, the number of ^{217}At decays in the kidneys was assumed equal to ^{221}Fr decays. The absorbed dose to the kidneys was obtained as the sum of the dose contribution from each daughter. The dose contribution from each daughter was calculated as the product of the number of decays per gram multiplied by the α-particle energy emitted per decay; photon and electron contributions to the absorbed dose were considered negligible relative to the α-particle dose.

Statistical analysis. Graphs were constructed using Prism (Graphpad software, Inc., San Diego, CA). Statistical comparisons between the experimental groups were done by either the Student's *t* test (two-group comparison) or one-way ANOVA with Bonferroni's multiple comparison post hoc test (three-group comparison). All statistical comparisons were two sided and the level of statistical significance was set at $P < 0.05$.

Results

Reduction in the renal ^{213}Bi activity by metal scavenging. The renal ^{213}Bi activity was significantly lower in DMPS- or DMSA-treated animals compared with untreated controls at 6 hours (ANOVA, $P < 0.0001$), 24 hours (ANOVA, $P < 0.0001$), and 72 hours (ANOVA, $P < 0.0001$) postinjection with the ^{225}Ac *in vivo* generator (Fig. 2*A*; Table 1). DMPS was significantly more effective than DMSA in preventing the renal ^{213}Bi accumulation at each time point (6 hours, $P < 0.001$; 24 hours, $P < 0.001$; 72 hours, $P < 0.001$). The renal ^{221}Fr activity, however, was not significantly different between the experimental groups at either 6 hours (ANOVA, $P = 0.39$), 24 hours (ANOVA, $P = 0.42$), or 72 hours (ANOVA, $P = 0.20$) postinjection. As shown in Fig. 2*B*, the mean blood ^{213}Bi activity was higher in the DMPS- and DMSA-treated groups compared with the controls (6 hours, ANOVA, $P < 0.0001$; 24 hours, ANOVA, $P < 0.0001$; 72 hours, ANOVA, $P < 0.0001$). Ca-DTPA treatment significantly prevented the renal ^{213}Bi accumulation (Fig. 2*C*), but it was less potent than DMPS in doing so [mean difference = 10.13; 95% confidence interval (95% CI), 5.48-14.77; $P < 0.0001$]. Similar experiments conducted up to 8 days postinjection with the radiolabeled antibody showed similar reduction (58.4%, 65.3%, and 60.7% at 120, 144, and 192 hours, respectively) in the renal bismuth activity, thus confirming the results obtained at early time points.

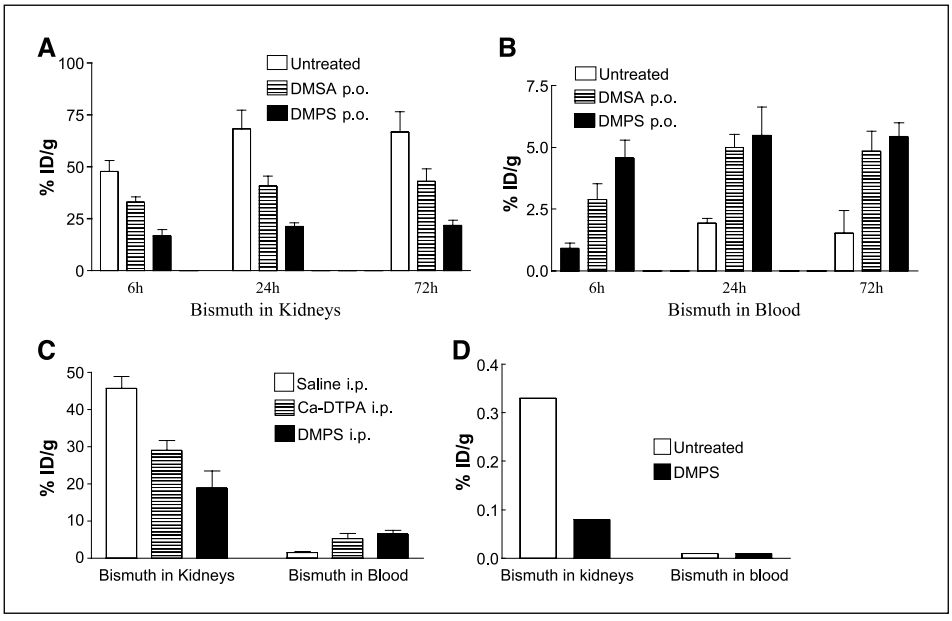

Figure 2. Effect of heavy metal chelators on ^{221}Fr and ^{213}Bi distribution in kidneys and blood. *A,* reduction in the renal ^{213}Bi activity by DMPS or DMSA treatment at 6, 24, and 72 hours postinjection. *B,* increase in the ^{213}Bi activity in blood by chelation therapy with DMPS or DMSA. *C,* comparison of the potencies of Ca-DTPA and DMPS in reducing in the renal ^{213}Bi accumulation at 24 hours postinjection. *D,* reduction in the 24-hour renal ^{213}Bi activity in a DMPS-treated cynomolgus monkey compared with an untreated animal. *Columns,* mean; *bars,* 95% CI. *%ID/g,* percentage of injected dose per gram of tissue.

Table 1. Reduction in the renal ^{213}Bi activity by chelation therapy

Group	Mean reduction	95% CI	*P*
6 h			
DMPS vs controls	31.15	25.91-36.38	<0.001
DMSA vs controls	14.82	9.59-20.05	<0.001
DMPS vs DMSA	16.33	11.09-21.56	<0.001
24 h			
DMPS vs controls	47.2	38.82-55.58	<0.001
DMSA vs controls	27.52	19.14-35.90	<0.001
DMPS vs DMSA	19.68	11.30-28.06	<0.001
72 h			
DMPS vs controls	45.04	35.33-54.75	<0.001
DMSA vs controls	23.74	14.03-33.45	<0.001
DMPS vs DMSA	21.3	11.59-31.01	<0.001

NOTE: Reduction in renal ^{213}Bi activity is expressed as mean difference in the percentage of injected dose per gram.

To test and confirm our hypothesis and result in an animal model more similar to humans, we also tested the effects of DMPS in a single monkey treated with DMPS compared with an untreated control monkey. The renal ^{213}Bi activity was lower in the DMPS-treated monkey (Fig. 2*D*) compared with the untreated one (0.08 and 0.33 %ID/g, respectively). However, the renal and blood ^{221}Fr and ^{225}Ac activities were unaltered by DMPS treatment (data not shown). Because this single experiment directly confirmed the results in mice, additional monkeys were not tested for ethical reasons.

Lack of an effect of hydration on ^{225}Ac daughter distribution and [^{225}Ac]HuM195 blood clearance. Renal activity of ^{213}Bi and ^{221}Fr was significantly lower at 192 hours compared with 24 hours postinjection (Fig. 3*A*). The renal distribution of ^{213}Bi and ^{221}Fr was not significantly altered by hydration at either 24 hours (^{213}Bi activity, mean difference = 2.78; 95% CI, −2.30-7.85; $P = 0.23$; ^{221}Fr activity, mean difference = 2.00; 95% CI, −1.05-5.05; $P = 0.16$) or 192 hours postinjection (^{213}Bi activity, mean difference = 3.90; 95% CI, −0.93-8.73; $P = 0.09$; ^{221}Fr activity, mean difference, 0.93; 95% CI, −0.84-2.70; $P = 0.23$). The blood ^{213}Bi and ^{221}Fr activity was also similar in the two groups (data not shown). After i.v. administration in mice, [^{225}Ac]HuM195 displayed a biphasic clearance from the blood with ~85% of the ^{225}Ac activity clearing rapidly within the first 24 hours and a slower clearance thereafter. The blood clearance of the labeled antibody was not significantly altered by hydration (Fig. 3*B*).

Prevention of renal ^{221}Fr and ^{213}Bi accumulation by diuretic therapy. In contrast to metal chelation, diuretic therapy prevented the renal accumulation of both ^{221}Fr and ^{213}Bi (Fig. 3*C*; Table 2). The 24-hour renal ^{213}Bi activity was significantly lowered by furosemide and chlorothiazide treatment (ANOVA, $P < 0.0001$). In addition, 24-hour renal ^{221}Fr activity was also significantly reduced (ANOVA, $P < 0.0001$) in furosemide- and chlorothiazide-treated groups compared with untreated controls. However, the renal ^{221}Fr and ^{213}Bi activities were not significantly different between the two diuretic treated groups (Bonferroni's post hoc analysis, $P > 0.05$ for both ^{221}Fr and ^{213}Bi activities; Fig. 3*C*; Table 2).

The combination of DMPS with a diuretic (furosemide or chlorothiazide) caused a greater reduction (~75-80%) in the renal ^{213}Bi activity than seen with DMPS or diuretic therapy alone (Fig. 3*D*; Table 3). The reduction in the renal ^{221}Fr accumulation, however, was similar to that seen with diuretic treatment.

Spironolactone, a potassium sparing diuretic, had no significant effect on the renal ^{221}Fr (mean difference = 1.84; 95% CI, −0.22-3.90; $P = 0.07$) and ^{213}Bi (mean difference = 2.56; 95% CI, −3.43-8.55; $P = 0.35$) accumulation compared with controls.

Effect of cold metal blockade on renal accumulation of ^{213}Bi. Competitive blockade of ^{213}Bi binding sites in the renal tubular cells by nonradioactive bismuth resulted in a moderate but significant reduction in the renal ^{213}Bi activity at both 6 hours (mean difference = 11.32; 95% CI, 4.81-17.83; $P = 0.004$) and 24 hours (mean difference = 16.70; 95% CI, 13.65-19.75; $P < 0.0001$) time points (Fig. 4*A*). As expected, the renal ^{221}Fr activity was unaltered at either time point (6 hours, $P = 0.10$; 24 hours, $P = 0.61$). In contrast, blockade of ^{213}Bi binding sites in blood by ferric

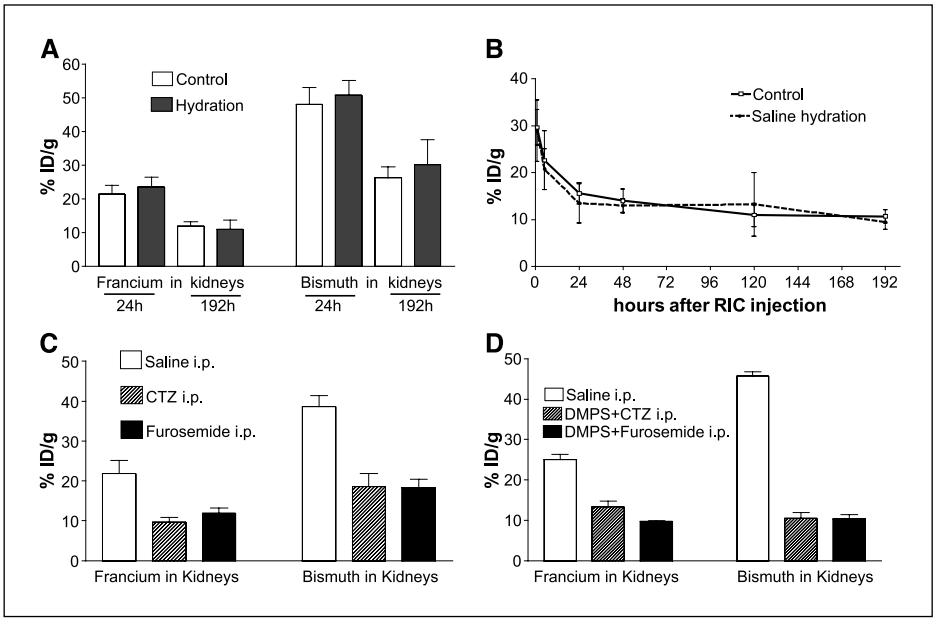

Figure 3. Effect of hydration, diuresis, or a combination of metal chelation and diuresis on renal ^{221}Fr and ^{213}Bi activity. *A,* renal ^{221}Fr and ^{213}Bi activity in saline hydrated or control mice at 24 and 192 hours postinjection. *B,* plasma clearance of the radioimmunoconjugate estimated by serial blood sampling from the retro-orbital venous plexus 1, 6, 24, 48, 120, and 192 hours (four to six animals per group at each time point) postinjection. Mice received i.p. saline or no treatment (controls). *C,* reduction in the 24-hour renal ^{221}Fr and ^{213}Bi activities by furosemide and chlorothiazide (*CTZ*) treatment. *D,* reduced renal accumulation of ^{221}Fr and ^{213}Bi at 24 hours postinjection by combination therapy with DMPS and furosemide or chlorothiazide. *Columns,* mean; *bars,* 95% CI.

Table 2. Reduction in the 24-hour renal ^{221}Fr and ^{213}Bi activities by diuretic treatment

Group	Mean reduction	95% CI	*P*
^{221}Fr			
Saline vs CTZ	12.28	9.97-14.58	<0.001
Saline vs furosemide	10.1	7.793-12.41	<0.001
CTZ vs furosemide	−2.18	−4.48-0.13	>0.05
^{213}Bi			
Saline vs CTZ	20.04	16.33-23.75	<0.001
Saline vs furosemide	20.36	16.65-24.07	<0.001
CTZ vs furosemide	0.32	−3.39-4.03	>0.05

NOTE: Reduction in the 24-hour renal ^{221}Fr and ^{213}Bi activities is expressed as mean difference in the percentage of injected dose per gram.
Abbreviation: CTZ, chlorothiazide.

ammonium citrate had no significant impact on 24-hour renal ^{221}Fr (P = 0.34) or ^{213}Bi (P = 0.28) activities (Fig. 4*B*).

Role of tumor burden in altering the pharmacokinetics of [^{225}Ac]SJ25C1 and ^{225}Ac daughters. We evaluated the role of tumor burden and the effect of DMPS in a disseminated Daudi lymphoma model system. The expression of CD19 and CD20 antigens and binding of the antibody (SJ25C1) to CD19 on Daudi cells were confirmed by flow cytometry before injecting the tumor cells in animals. Flow cytometric analysis was done in a single animal to show that the tumor growth occurred within this time period. The percentage of target lymphoma cells (bone marrow cells positive for human CD20) in one representative low-burden mouse was 0.12% and was 27% in a high-burden animal. Five animals were examined for biodistribution in each group. Due to higher localization of the labeled antibody (^{225}Ac activity) to the lymphoma cells in the femurs (Fig. 5*A*), the kidney-to-femur activity ratio for ^{225}Ac was significantly lower in the group with higher tumor burden compared with low tumor burden group (mean difference = 0.57; 95% CI, 0.31-0.83; P < 0.0001; Fig. 5*B*). As shown in Fig. 5*C*, the presence of a higher tumor burden resulted in a significant decrease in the renal ^{213}Bi activity (mean difference = 13.68; 95% CI, 4.40-22.96; P = 0.003). This amount was further reduced by DMPS treatment (mean difference = 22.26; 95% CI, 12.98-31.54; P < 0.001 compared with untreated high-burden group and mean difference = 35.94; 95% CI, 26.66-45.22; P < 0.001 compared with low-burden group). However, DMPS also reduced the ^{213}Bi activity in the femurs (by 43.1%) to a slightly lower degree than in the kidneys (by 58.4%). The femur ^{213}Bi activity was significantly higher in the untreated high-burden group compared with the low-burden group (mean difference = 5.82; 95% CI, 3.87-7.77; P < 0.001). The ratio of kidney to femur activity for ^{213}Bi was significantly lower in the groups with higher tumor burden (low-burden versus untreated high-burden group, mean difference = 15.33; 95% CI, 11.80-18.86; P < 0.0001; Fig. 5*B*).

Table 3. Reduction in the 24-hour renal ^{221}Fr and ^{213}Bi activities by a combination of chelation and diuretic therapy

Group	Mean reduction	95% CI	*P*
^{221}Fr			
Saline vs DMPS + CTZ	11.7	7.80-15.60	<0.001
Saline vs DMPS + furosemide	15.3	11.40-19.20	<0.001
DMPS + CTZ vs DMPS + furosemide	3.6	−0.30-7.50	>0.05
^{213}Bi			
Saline vs DMPS + CTZ	35.91	31.66-40.16	<0.001
Saline vs DMPS + furosemide	35.99	31.74-40.24	<0.001
DMPS + CTZ vs DMPS + furosemide	0.08	−4.17-4.34	>0.05

NOTE: Reduction in the 24-hour renal ^{221}Fr and ^{213}Bi activities is expressed as mean difference in the percentage of injected dose per gram.

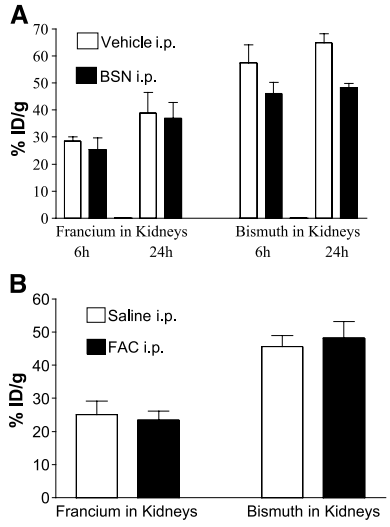

Figure 4. Effect of competitive metal blockade on ^{225}Ac daughter distribution. *A,* reduction in the renal ^{213}Bi activity by bismuth subnitrate (*BSN*) at 6 and 24 hours postinjection. *B,* ^{221}Fr and ^{213}Bi activity in the kidneys of control and ferric ammonium citrate (*FAC*)–treated animals at 24 hours postinjection. *Bars,* 95% CI.

Renal dosimetry. The estimated absorbed dose to mice kidneys after injection with 0.35 μCi [^{225}Ac]HuM195 is 27.6 Gy; with absorbed dose contributions of 0.6, 7.1, 7.9, and 12 Gy from ^{225}Ac, ^{221}Fr, ^{217}At, and ^{213}Bi, respectively. The estimated renal dose following chelation therapy is 19.6 Gy due to a reduction in the ^{213}Bi dose from 12 to 4 Gy. Diuresis with chlorothiazide or furosemide reduces the renal dose estimates from ^{221}Fr, ^{217}At, and ^{213}Bi to 3.2, 3.6, and 6 Gy, respectively (total dose = 13.4 Gy). A combination of chelation and diuresis reduces the estimated renal absorbed dose to 9 Gy with contributions from ^{221}Fr, ^{217}At, and ^{213}Bi being 2.8, 3.1, and 2.5 Gy, respectively.

Discussion

Radioimmunotherapy has advanced tremendously in the last 20 years with the development of more sophisticated carrier molecules and radionuclides with emission characteristics, half-lives, and chemical properties suited to a particular cancer and therapeutic application (26, 27). High–linear energy transfer, short path length

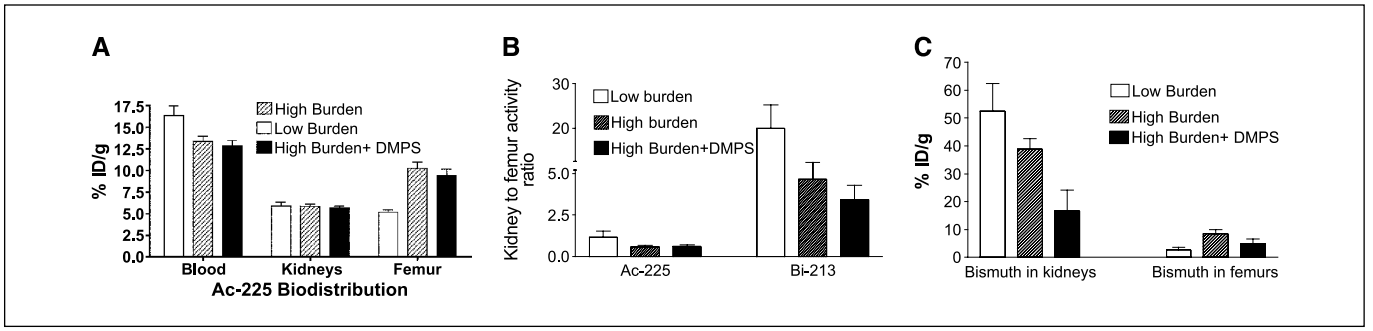

Figure 5. Effect of tumor burden on ^{225}Ac daughter distribution. *A,* alteration in the distribution of ^{225}Ac-labeled antibody in animals with a higher tumor burden compared with those with lower tumor burden. *B,* reduction in the ratio of kidney to femur activity for ^{225}Ac and ^{213}Bi in animals with higher versus lower tumor burden. DMPS treatment further reduced the kidney-to-femur activity ratio for ^{213}Bi. *C,* reduction in the renal ^{213}Bi activity by the presence of higher tumor burden and further enhancement of the effect by concomitant DMPS treatment. *Bars,* 95% CI.

α-particles can mediate efficient and selective cell kill with little nonspecific cytotoxicity (2–6, 28, 29). The results of preclinical studies with antibody-targeted, α-particle–emitting ^{225}Ac *in vivo* generators have generated optimism as well as caution for their potential human clinical use (8, 9). The advantage of using ^{225}Ac is its relatively long half-life (10 days) compared with other α-particle–emitting radionuclides used for radioimmunotherapy in humans (^{213}Bi, $t_{1/2}$ = 45.6 minutes; ^{211}At, $t_{1/2}$ = 7.2 hours; refs. 29, 30). This permits delivery of radiation even to the less readily accessible cells (8).

^{225}Ac decays sequentially through three α-particle–emitting daughters (^{221}At, ^{217}Fr, ^{213}Bi) to yield a net of four α-particles. Although this property greatly enhances the potency of the ^{225}Ac *in vivo* generators, it also results in increased toxicity as the systemically released radioactive daughters may get transported to and irradiate the normal tissues. The ^{225}Ac immunoconjugate is stable *in vivo* and the daughters released inside the target cell remain internalized (8). However, the daughters released from the circulating ^{225}Ac *in vivo* generator tend to distribute independently of the parent construct (10).

In the model here, we picked kidneys as the primary target organ for our studies for two reasons. First, ^{213}Bi, potentially the most toxic ^{225}Ac daughter, tends to accumulate preferentially in the kidneys (15–17, 19). In addition, francium, an alkali metal like sodium and potassium, can possibly accumulate in renal tubular cells. Second, the results from our toxicity studies in monkeys injected with high doses of antibody-^{225}Ac suggest delayed nephropathy progressing to renal failure as the cause of severe morbidity in these animals (10).

We tested several pharmacologic approaches and the role of tumor burden in preventing the renal accumulation of ^{225}Ac daughter nuclides. The dithiol chelators, DMPS and DMSA, are known to chelate and enhance the urinary excretion of bismuth (12, 18–20,). Ca-DTPA has been used in the United States as a chelating agent for plutonium and other transuranic elements (31, 32). In our studies, each of these chelators significantly reduced the renal ^{213}Bi accumulation. However, DMPS was the most efficacious agent at all the time points.

In human plasma, DMPS has been shown to form nonprotein sulfhydryls to a greater extent (37%) than DMSA (8%; ref. 33). Therefore, DMPS is thought to be more reactive in plasma than DMSA (33). This may account for the greater effectiveness of DMPS in the studies here in reducing the renal ^{213}Bi uptake compared with DMSA. DMPS gets rapidly oxidized in aqueous solutions to form disulfides (34). We, however, did not see a loss of efficacy when it was administered in drinking water compared with parenteral administration. This is possibly due to disulfide reduction in the renal tubular cells by a glutathione-disulfide exchange reaction to yield the parent drug (33).

The mechanism for the increase in the blood ^{213}Bi activity with chelation therapy may be related to the chelation and retention of ^{213}Bi generated in blood from the circulating ^{225}Ac *in vivo* generators or the extraction of tissue ^{213}Bi into the blood stream. However, we did not see an increase in the whole body retention of ^{213}Bi. The consequences of increased blood dose are not known. The circulating chelator-^{213}Bi complex may not cause any significant toxicity due to the short path length of α-particles. In the clinical trial with ^{213}Bi-labeled HuM195, α-particle irradiation in blood was not associated with significant toxicity for injections of up to 100 mCi ^{213}Bi (30). In contrast, any reduction in the renal ^{213}Bi activity is likely to improve the safety of the ^{225}Ac *in vivo* generators.

Although hydration can increase the renal plasma flow and glomerular filtration rate via an increase in the intravascular volume, the filtered load (product of the glomerular filtration rate and the plasma concentration) of ^{213}Bi and ^{221}Fr could remain unchanged as the plasma concentrations of the daughters decrease with an increase in blood volume. Therefore, in the absence of an intervention to limit the tubular reabsorption of the ^{213}Bi and ^{221}Fr, their excretion was not enhanced by hydration alone.

Different classes of diuretics inhibit the tubular reabsorption of the alkali metals, Na^+ or K^+ or both, although they differ in their potency, mechanism, and site of action within the nephron. Furosemide and chlorothiazide act in the ascending limb of Henle's loop and distal convoluted tubule of the nephron, respectively (35). The significant drop in the renal ^{221}Fr activity with furosemide and chlorothiazide is possibly due to an inhibition of the renal tubular reabsorption of ^{221}Fr, which is an alkali metal and is, therefore, expected to behave like Na^+ and K^+. Because ^{213}Bi is generated from ^{221}Fr, a decrease in the renal ^{213}Bi ensued. Furthermore, the combination of DMPS with a diuretic (furosemide or chlorothiazide) resulted in an even greater reduction in renal ^{213}Bi activity than seen with DMPS or the diuretics alone. The absence of an effect of spironolactone, a potassium-sparing diuretic, on renal ^{213}Bi and ^{221}Fr activity can be explained by its low potency (35). The administered doses of furosemide and chlorothiazide were scaled from previously published literature on their ED_{50} in mice. The doses exceed the human therapeutic doses as there is a species difference in the ED_{50} of these drugs (36).

Bismuth is both filtered as well as secreted because the renal clearance of bismuth is greater than that of inulin (15). It is believed that ionized bismuth (Bi^{3+}) is bound to the plasma

proteins and forms a slow-clearing compartment (15). We attempted to competitively limit the number of available binding sites for ^{213}Bi on transferrin (37) by use of ferric ammonium citrate. This strategy was expected to increase the amount of unbound ^{213}Bi in the plasma and, therefore, accelerate its excretion via kidneys. The absence of a significant enhancement of ^{213}Bi excretion may be due to the binding of the ^{213}Bi to albumin (38) when the binding sites on transferrin were blocked by ferric ammonium citrate. In contrast, competitive blockade of either ^{213}Bi uptake or its binding sites (metallothionein-like proteins) in the renal tubular cells (16, 17) by nonradioactive bismuth subnitrate significantly reduced the accumulation of ^{213}Bi in the kidneys at both 6 and 24 hours postinjection.

The daughters released after internalization of the labeled antibody are retained inside the target cells (8). The presence of high levels of a specific target (tumor burden) caused a decrease in the amount of circulating, untargeted antibody and, therefore, the systemically released daughters. This translated to an increase in the activity of ^{225}Ac and its radioactive daughters in the femurs (where the tumor resided) and a corresponding decrease in their activities in the kidneys. This positive effect may have been blunted by the large dose of antibody used and the low specific activity of the radioimmunoconjugate as ~1 of 1,000 antibodies were labeled with ^{225}Ac. Based on the number of available CD19 sites per Daudi cell, 120×10^6 tumor cells (estimated tumor load in a high-burden animal) are expected to maximally absorb ~1.2 μg of the antibody, whereas 6.7 μg of the antibody was injected per animal. This translates to an excess of injected antibodies compared with the available binding sites. In contrast, in a typical acute myeloid leukemia patient with ~10^{12} leukemia cells, ~5 mg HuM195 could be absorbed, which is a typical dose of this antibody in humans (30). At these antibody doses, a more pronounced reduction in the renal daughter accumulation could be expected.

DMPS treatment further reduced the renal ^{213}Bi accumulation in animals that bore the target tumor. Additionally, a reduction in the femur ^{213}Bi activity was seen in these animals. However, despite the reduction in the ^{213}Bi activity in the femurs, the kidney-to-femur activity ratio in these animals for ^{213}Bi was significantly lower. This is because of a greater relative reduction in the ^{213}Bi accumulation in kidneys than in the femurs. Free bismuth has been shown to accumulate in the femurs even in the absence of a bone marrow tumor (12). Therefore, the ^{213}Bi activity in the femurs cannot be entirely accounted for by the ^{213}Bi inside the tumor cells. The reduction in the femur ^{213}Bi activity may be due to its scavenging from the tumor cells or the femurs. It could also be due to scavenging of free ^{213}Bi produced on the surface of the tumor cells as a result of the attachment of the labeled antibody. Furthermore, three α-particle emissions would have occurred in the target cell before ^{213}Bi is formed. However, a possible reduction in the antileukemia activity is possible.

The doses of DMPS used in our studies are in the recommended 0.1 to 1 mmol/kg/d range for the treatment of heavy metal poisoning (12). Although the effective half-lives of many radiolabeled antibodies in humans are usually measured in several days, the 10-day half-life of ^{225}Ac and continuous generation of daughters may require that patients treated with ^{225}Ac *in vivo* generator be administered chelation therapy for a relatively longer period. DMPS has been shown to be safe when used long term (months) for heavy metal detoxification trials in humans (39, 40). The interexperimental variance in the tissue daughter activities at a given time point was expected owing to possible age-related variability in the capacity of the reticuloendothelial system to metabolize the labeled antibody. However, the intraexperimental variability within an experimental group was small.

A uniform distribution of α-particle energy was assumed in the calculations for the renal absorbed dose. In the absence of detailed microdistribution data, these estimates are an oversimplification considering the high energy and short path-length of α-particles. The results are presented in units of Gy rather than Sv because the exact relative biological effectiveness value of α-particles for kidney cells *in vivo* is not known. Radiation-induced decrease in renal function is a late change and may take months to years to manifest (41). In comparison with other species, mouse kidneys are relatively radioresistant and exhibit radiation-induced renal injury at a much later time point (41). Therefore, the impact of these pharmacologic interventions on long-term survival is difficult to assess in mice. However, because the development of radiation nephropathy is dose-dependent (41), we speculate that a reduction in the radiation dose to the kidneys might be beneficial. Proof of this hypothesis will require long-term primate or human studies, which are planned.

In summary, we tested the value of different pharmacologic agents and the role of tumor burden in altering the pharmacokinetics of the ^{225}Ac-labeled antibody and the released decay daughters. The results suggest that chelation and diuretic therapy, alone or in combination, and competitive metal blockade to a certain extent can reduce the renal accumulation of ^{225}Ac daughters. Therefore, these approaches could possibly enhance the therapeutic index of the ^{225}Ac immunoconjugates. The findings support the use of dithiol chelators, thiazide diuretics, and furosemide as possible adjuvants for future clinical trials with ^{225}Ac *in vivo* generators.

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