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Comparison of ^{225}Actinium Chelates: Tissue Distribution and Radiotoxicity

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ABSTRACT. The biodistribution and tissue toxicity of intravenously administered 225-actinium (^{225}Ac) complexed with acetate, ethylene diamine tetraacetic acid (EDTA), 1, 4, 7, 10, 13-pentaazacyclopentadecane-N, N′, N″, N‴, N⁗-pentaacetic acid (PEPA), or the "a" isomer of cyclohexyl diethylenetriamine pentaacetic acid (CHX-DTPA), were examined. The percent of injected dose per organ and per gram of tissue for each chelate complex was determined. ^{225}Ac-CHX-DTPA was evaluated further for radiotoxic effects. Mice receiving ≥185 kBq ^{225}Ac-CHX-DTPA suffered 100% morbidity by 5 days and 100% mortality by 8 days postinjection, and all animals evaluated had significant organ damage. The *in vivo* instability of the ^{225}Ac-CHX-DTPA complex likely allowed accumulation of free ^{225}Ac in organs, which resulted in tissue pathology. NUCL MED BIOL 26;5:581–589, 1999. **© 1999 Elsevier Science Inc. All rights reserved.**

KEY WORDS. α-Particle, Biodistribution, Radiotoxicity, Tissue toxicity, Biologic effective dose, Chelators

INTRODUCTION

Alpha-particle (α) emitting radioisotopes are uniquely suited to localize therapy to within 100 μm of suitable targets. The α-emitters, ^{211}Astatine (At) ($t_{½}$ = 7.2 h), ^{212}Lead (Pb) ($t_{½}$ = 10.6 h), ^{212}Bismuth (Bi) ($t_{½}$ = 60.6 m), and ^{213}Bi ($t_{½}$ = 45.6 m), are currently being tested for therapeutic efficacy *in vivo* (18, 21) and ^{211}At, ^{212}Bi, and ^{213}Bi have recently entered Phase I and II clinical trials (21). ^{225}Actinium (Ac), a daughter of the ^{233}Uranium (Ur) decay chain, is an α-emitter that has gained recognition recently because of its convenient half-life (10 days), its release of 4 α particles per ^{225}Ac atom, and its ability to serve as a generator for other radionuclides (8, 18). Thus, the therapeutic potential of ^{225}Ac requires further investigation.

Chelators are used to complex radiometals and couple them to targeting molecules, thus minimizing potential nonspecific tissue damage. Limited information exists regarding the behavior of ^{225}Ac *in vivo*. Preliminary studies have evaluated ^{225}Ac complexed to citrate with respect to tissue uptake, biodistribution, and tumor tropism in animal models (6, 15, 24). Previous studies using ^{225}Ac complexed to either of the polyaminocarboxylate chelators, ethylene diamine tetraacetic acid (EDTA), or cyclohexyl diethylenetriamine pentaacetic acid (CHX-DTPA) showed varied tissue tropism and elevated blood clearance compared with uncomplexed ^{225}Ac (4). Interestingly, ^{225}Ac-CHX-DTPA-monoclonal antibody (Mab) complexes used to determine biokinetic behavior on tumor-bearing nude mice showed successful *in vitro* complexing but poor stability *in vivo* (4). Thus, whereas ^{225}Ac may prove useful in radiotherapeutic models, information regarding potentially effective chelators and the relative stability of such ^{225}Ac complexes *in vivo* is lacking. The goals of this study were to determine the distribution and toxicity of ^{225}Ac chelated to various compounds and the potential usefulness of one such compound that appears to be the most promising, ^{225}Ac-CHX-DTPA, in radiotherapy models. The chelating ligands used included EDTA, CHX-DTPA, and 1, 4, 7, 10, 13-pentaazacyclpentadecane-*N*, *N′*, *N″*, *N‴*, *N⁗*-pentaacetic acid (PEPA). The biodistributions of these were compared with that for ^{225}Ac-acetate. ^{225}Ac-CHX-DTPA was used to determine the maximum tolerated dose (MTD). ^{225}Ac-CHX-DTPA-Mab 201B was used for toxicity studies, including acute and chronic effects.

MATERIALS AND METHODS

Synthesis of Chelators: CHX-DTPA and PEPA

The "a" isomer of CHX-DTPA was synthesized and used as reported previously (13, 19). 1, 4, 7, 10, 13-Pentaazacyclopentadecane pentasulfate was synthesized by the published method (2) and the free base prepared. The free base (1.6 mmol) was treated with *tert*-butyl bromoacetate (23.8 mmol) and Na_2CO_3 (18.9 mmol) in CH_3CN (5 mL) and stirred for 10 days, followed by purification by elution with 0–10% $CH_3OH/CHCl_3$ on silica. The pentaester was heated at 105°C for 15 h with 12 M HCl. After removal of the acid, PEPA was recovered in good yield (80%). All spectra agreed with the literature values (16).

Radionuclide Preparation

^{225}Ac was separated from ^{225}Radium (Ra) ($t_{½}$ = 15 days) by ion exchange and extraction column chromatography as described previously (5). Stock solutions of purified ^{225}Ac in 0.1 M HNO_3 were freshly prepared as needed. Complexes of ^{225}Ac with the chelators EDTA, CHX-DTPA, and PEPA were prepared by mixing ~100 μL ^{225}Ac solution (~10 MBq in 0.1 M HNO_3) with 20 μL

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of chelator (~1.0×10^{-2} M in H_2O) and adjusting the pH to near 5.0 by the addition of 5–10 μL of 1.0 M NH_4OAc. The mixture was kept at 40°C for 30 min, then purified on a Chelex column (~300 μL bed volume, pre-equilibrated with 1.0 M NH_4OAc, pH = 5.0). Typically, 80–90% of activity was eluted, representing complexed species, from the Chelex column with 2 mL NH_4OAc reagent. ^{225}Ac-acetate was not passed over the Chelex columns because of the high affinity of the column resin for cationic species.

Chelated ^{225}Ac mixtures were diluted in 2-[N-morpholino]ethanesulfonic acid (MES) buffer, pH = 6.2, to final concentrations of 92, 185, 370, or 740 kBq in 200 μL of solution, which was injected intravenously into mice. Aliquots were taken from each dilution to serve as standards for activity determination. Stored chelate complexes were also evaluated 24 h after chelation to confirm stability. These samples were passed through a 0.2-μm filter and then aliquots of each filtrate were applied to Chelex columns. In all cases, >95% of ^{225}Ac was found complexed to its ligand.

^{225}Ac-CHX-DTPA-Mab 201B

Mab 201B binds to thrombomodulin on mouse lung endothelial cells and has been used for effectively targeting radioisotopes to lung (13). Mab 201B was coupled with CHX-DTPA as described previously (13) and was incubated for 30 min with ^{225}Ac in sodium acetate buffer (pH = 5.5). The mixture was separated on size exclusion high performance liquid chromatography (HPLC) in MES buffer (pH = 6.8) at 1 mL/min flow as described elsewhere (19); the included (IgG) fraction contained both ^{225}Ac and ^{213}Bi as expected. Retention of the ^{225}Ac-DTPA Mab 201B complex by a Microcon-30 filter analysis (13) indicated approximately 93% coupling of this complex.

Animals

Female BALB/c mice were purchased from Taconic Farms (Germantown, NY) and housed under specific pathogen-free conditions at the Oak Ridge National Laboratory. Animal studies were conducted according to an institutionally ACUC approved protocol, #0204, following IACUC guidelines. For most studies described herein, mice were injected between the ages of 8 and 10 weeks. All injections were performed in laminar flow hoods and postinjection, animals were returned to sterilized cages and had free access to food and water.

Distribution Studies

To determine the organ distribution of the various ^{225}Ac complexes, mice were injected via a tail vein with a total volume of 200 μL MES buffer containing 92 kBq of each ^{225}Ac complex. Distribution was determined in three to five mice at 1 h, 4 h, 24 h, 5 days, and 8 days postinjection. After sacrifice, the following samples were collected from each mouse and placed into previously tared scintillation vials: skin, muscle, femur, reproductive tract (uterus and ovaries), stomach (emptied of ingesta), jejunem, ascending colon, liver, kidney, spleen, sternum, thymus, heart, lung, and 10 μL of whole blood. Weights and the ^{225}Ac content of each sample were determined 4 h after collection (to allow for ^{213}Bi/^{225}Ac equilibration) using a NaI (Tl) γ-scintillation counter. Three windows were set on the γ counter to measure ^{225}Ac, ^{213}Bi, and a combination of the two radioisotopes. A lower energy window was set to measure the 188 and 218 keV γ-rays from ^{225}Ac and ^{221}Francium (Fr, immediate daughter of ^{225}Ac, $t_{½}$ = 4.8 min, see Table 4), respectively. The 440-keV γ-ray from ^{213}Bi was measured in the upper energy window. All tissue sample values are presented as percent injected dose per gram (%ID/g) of tissue ± SD. Whole organ ^{225}Ac content (%ID/organ) was then determined for select organs based on the above values and the percent of body weight accounted for by each organ. Absorbed dose values for ^{225}Ac were estimated using calculation techniques described previously (11).

MTD Studies

Mice were injected as described above with ^{225}Ac-CHX-DTPA at doses of 92, 185, 370, and 740 kBq in a total of 200 μL MES buffer to determine the MTD for survival. Control mice received only CHX-DTPA in 200 μL MES buffer IV. MTD was determined by monitoring clinical signs and grading pathological changes in organs following sacrifice and by monitoring total peripheral white blood cell (WBC) counts, body weight, and the time to development of clinical signs of radiation-induced illness that necessitated sacrifice. Mice were considered clinically ill when they demonstrated weight loss, ceased self-grooming, separated themselves from cage mates, or had difficulty walking. Mice were killed by cervical dislocation when a 20% or greater loss in body weight or neurologic signs developed, or when they were moribund. Neurologic signs were not limited to but predominantly consisted of vestibular disturbances.

Toxicity Studies as Assessed by Organ System Damage

To determine both the acute and chronic effects of ^{225}Ac-CHX-DTPA at the administered doses of 92, 185, 370, and 740 kBq, two mice from each group were killed at days 2, 5, and, if not previously sacrificed due to clinical signs of radiation toxicity, days 7 and 53 postinjection. Mice receiving only intravenous CHX-DTPA in MES buffer served as controls. After sacrifice, lungs were inflated with 10% buffered neutral formalin and all tissues suspended in this fixative for 24 h at room temperature. Tissues were then rinsed and placed in phosphate-buffered saline (PBS) at 4°C until paraffin embedding could be performed. Embedded tissues were cut to a thickness of 6 μm, stained with hematoxylin and eosin, and permanently mounted. All tissues from all mice were evaluated individually for evidence of damage, attributable to radiation, which was graded as mild, moderate, or severe under the guidance of a board-certified veterinary pathologist.

Determination of MTD by Peripheral Total WBC Counts

Mice receiving ^{225}Ac-CHX-DTPA at doses of 92, 185, 370, and 740 kBq and control mice receiving CHX-DTPA in MES were anesthetized with Metofane (Pittman-Moore, Mundelein, IL) and whole blood was collected via puncture of the orbital venous sinus with heparinized capillary tubes. Total WBC counts were determined 2 days before and 2 and 5 days postinjection using a hemocytometer. Whole blood was diluted 1:20 in a solution consisting of 1 part 1% Gentian Violet, 1 part glacial acetic acid, and 98 parts distilled water. Differential leukocyte counts were not determined.

RESULTS

Organ Distribution of ^{225}Ac Complexes

The amount of injected ^{225}Ac retained in select tissues was determined following intravenous administration and values for %ID/g (Fig. 1) of tissue ± SD were calculated. On a per gram basis,

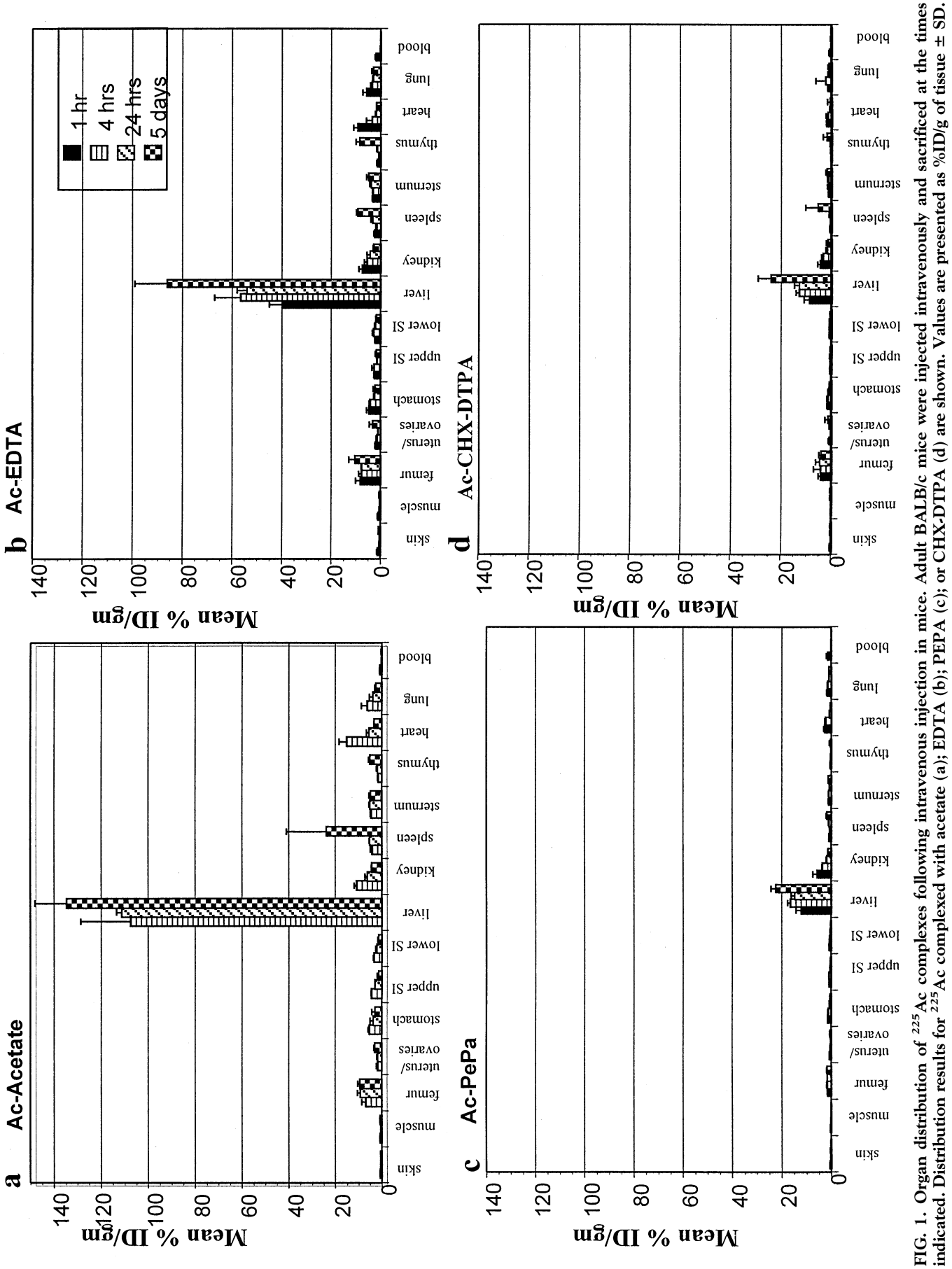

FIG. 1. Organ distribution of ^{225}Ac complexes following intravenous injection in mice. Adult BALB/c mice were injected intravenously and sacrificed at the times indicated. Distribution results for ^{225}Ac complexed with acetate (a); EDTA (b); PEPA (c); or CHX-DTPA (d) are shown. Values are presented as %ID/g of tissue ± SD.

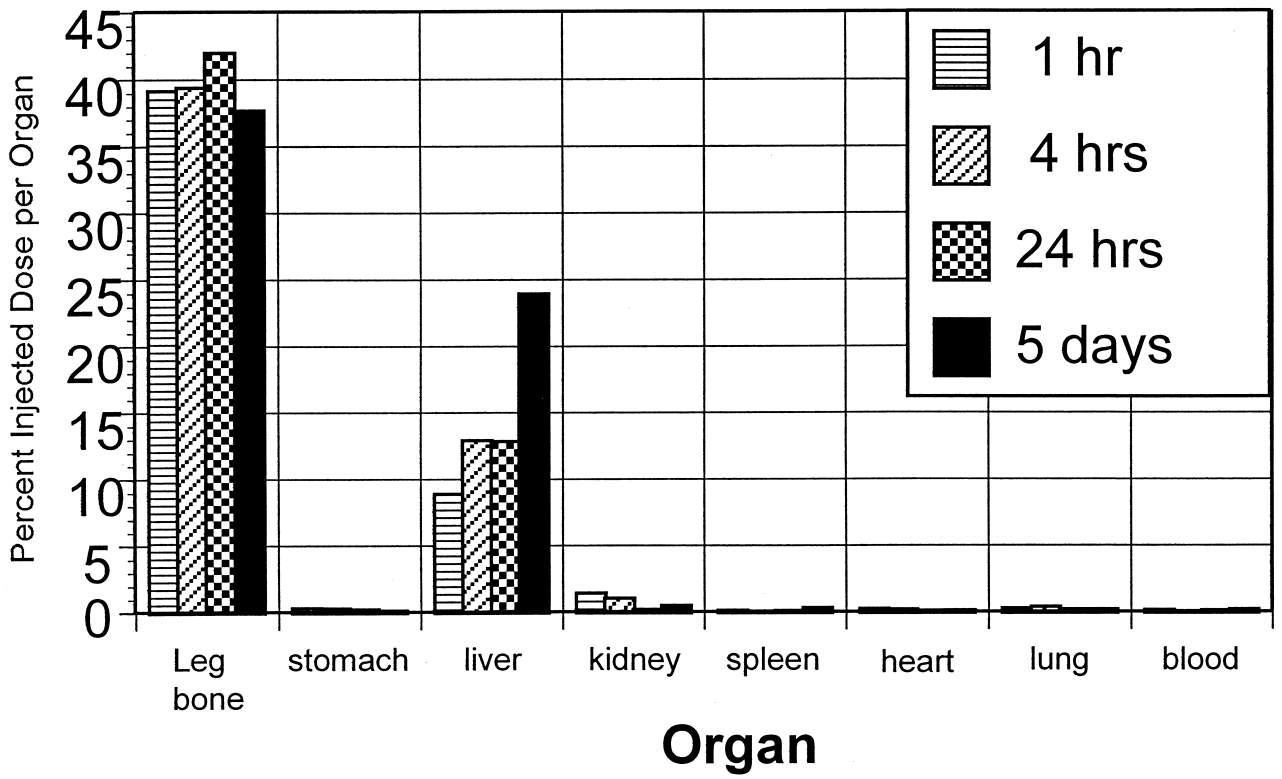

FIG. 2. Organ distribution of ^{225}Ac-CHX-DTPA following intravenous injection in mice. Adult BALB/c mice were injected intravenously with 200 μL of ^{225}Ac-CHX-DTPA (92 kBq) and animals (3–5/group) were sacrificed at the times indicated. Values are presented as %ID/organ.

^{225}Ac distributed predominantly to the liver, with lesser amounts to the skeleton (femur), kidney, and heart (Fig. 1). Increased retention was seen in the spleen at later time points; however, the measured values showed wide variation and may, in fact, have reflected extremely low splenic weights at these time points due to toxicity to leukocytes. Interestingly, when expressed as %ID/organ, all ^{225}Ac compounds had a predominant distribution to bone. Figure 2, which presents %ID/organ data for ^{225}Ac-CHX-DTPA, reveals the organ distribution patterns that were common to all ^{225}Ac compounds. Accumulation and retention within the liver of ^{225}Ac-Acetate (Fig. 1a) was significantly higher at all time points than any of the chelated complexes. Specifically, the %ID/g for liver of ^{225}Ac-Acetate was more than 1.5 times that for ^{225}Ac-EDTA and more than 5.5 times that for ^{225}Ac-CHX-DTPA and ^{225}Ac-PEPA at all time points (Figs. 1 and 2). Although values for liver and bone increased with time, distribution of all forms of ^{225}Ac to the kidney and heart decreased over time, reflecting temporal clearance from these organs. These comparisons are further illustrated in Figure 3, in which the %ID/g of the injected ^{225}Ac complexes are presented for individual organs. The data show that while ^{225}Ac, in all chelated forms examined in this study, distributed predominantly to the liver, femur, and kidney, the amount retained in these organs was greatly reduced when CHX-DTPA or PEPA were used as the chelating ligands. An alternate chelating ligand, DOTA (1, 4, 7, 10 tetraazacyclododecane-tetraacetic acid) is currently being investigated in our laboratory and when coupled to ^{225}Ac, appears to be somewhat more stable than ^{225}Ac-PEPA, although toxicity studies are pending (unpublished data).

Determination of Delivered Dose of ^{225}Ac

Alpha particle absorbed energy doses were calculated for various organs using a mouse model (11, 25) and radioisotope distribution data from this work. Doses are expressed as Gy per injected dose (92 kBq) of ^{225}Ac and were calculated for ^{225}Ac-EDTA and ^{225}Ac-CHX-DTPA only. These data, presented in Table 1, confirm the superior stability of ^{225}Ac-CHX-DTPA, because organ absorbed doses with this complex are less than half of those observed following ^{225}Ac-EDTA injection at this injected dose. The dose to liver was 7.6–34.9 times more than that to other organs with ^{225}Ac-EDTA and 3.9–27.6 times more with ^{225}Ac-CHX-DTPA.

^{225}Ac-DTPA-Mab 201B Biokinetics

To test the effective *in vivo* stability of the ^{225}Ac-CHX-DTPA complex, an animal model was used. Mab 201B reacting with murine thrombomodulin had been shown (12, 13) to target chiefly to murine lung endothelium and remain bound there for several days ($t_{½}$ ~5 days). CHX-DTPA-Mab 201B (11) complexed with ^{225}Ac and purified using HPLC was injected intravenously into test animals. Distribution data presented in Figure 4 indicated that even though the complex homes chiefly to the lung as expected, ^{225}Ac was lost rapidly from Mab 201B. This finding is in contrast to the behavior of the ^{213}Bi-CHX-DTPA-Mab 201B complex or with the values for ^{125}I Mab (10, 11). At the 24-h sacrifice, tissue was tested immediately after collection for the ratio of ^{213}Bi to ^{225}Ac, as determined by their characteristic γ-rays, 440 and 218 keV, respectively (218 keV actually emitted from ^{221}Fr, the daughter of ^{225}Ac,

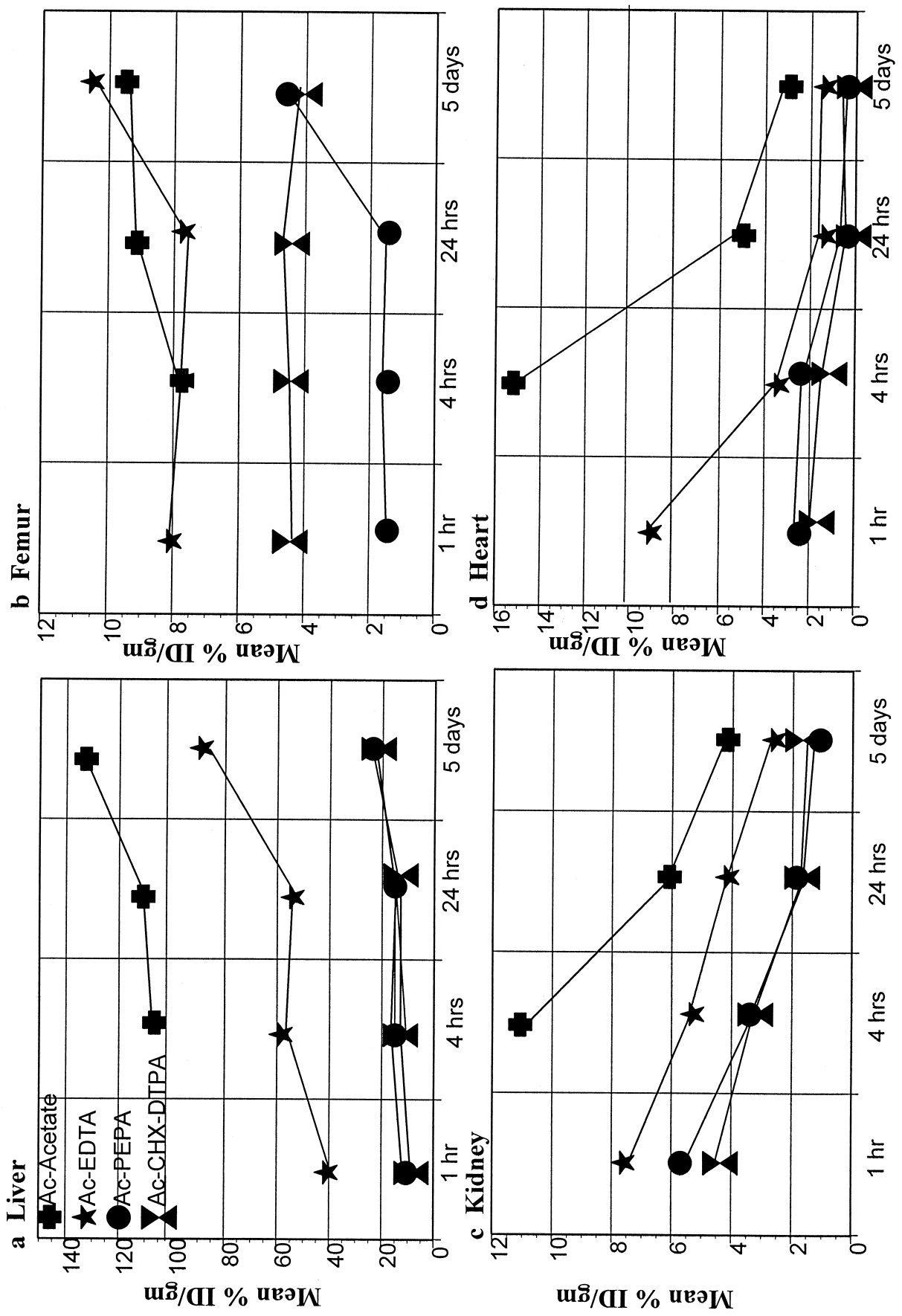

FIG. 3. Specific organ distribution of ^{225}Ac-CHX-DTPA following intravenous injection. BALB/c mice were injected as for Figure 1. Values presented are %ID/g of liver (a), bone (b), kidney (c), and heart (d) ± SD.

TABLE 1. α Particle Absorbed Dose to Organs From 225-Ac

Organ	^{225}Ac-EDTA	^{225}Ac-CHX-DTPA
Liver	117.8	30.4
Bone	15.4	7.8
Spleen	9.6	4.4
Kidney	2.1	3.2
Lungs	5.3	2.0
Heart	3.4	1.1
Uterus/ovaries	3.4	1.3

All doses are expressed as Gy per injected dose of 92 kBq.

see Table 4). The lung contained significantly lower levels of ^{213}Bi than ^{225}Ac (^{213}Bi/^{225}Ac ratio = 0.61), whereas kidney contained higher levels of ^{213}Bi (ratio 2.64). A near equilibrium amount of the radioisotopes was found in the liver (ratio 0.92). These data indicate that when ^{225}Ac-CHX-DTPA-Mab 201B decays in the lung, it releases ^{213}Bi into the blood stream and the ^{213}Bi subsequently accumulates in the kidneys. Further, these data indicate that the release of ^{225}Ac from the Mab 201B chelate complex would result in a relatively low dose to the targeted organ, and could result in damage to normal tissue (liver and gastrointestinal [GI] tract).

^{225}Ac-CHX-DTPA Effect on WBC

Studies to determine the MTD and organ toxicity were performed with ^{225}Ac-CHX-DTPA because of its relative stability. Total peripheral WBC counts served as a general screening for animal health and are presented in Figure 5. Significant differences were seen between preinjection WBC counts and those at 2 and 5 days postinjection for all ^{225}Ac-CHX-DTPA doses and mice receiving "cold" CHX-DTPA in MES. The acute decrease in WBC counts suggests that one toxic effect of ^{225}Ac-CHX-DTPA is on circulating leukocytes, which is supported by the decrease in total splenic weights of mice receiving 92 kBq of ^{225}Ac-CHX-DTPA (Table 2). Additionally, as discussed below, bone marrow toxicity was observed in all ^{225}Ac-CHX-DTPA-treated mice reflecting, perhaps, an increased susceptibility to α-radiation in cells that have either higher metabolic rates or are undergoing cell division.

The decreased WBC counts in mice receiving 92 kBq of ^{225}Ac-CHX-DTPA, a dose that was tolerated by mice and associated with no morbidity or mortality, was accompanied by a decrease in mean splenic weights, as shown in Table 2. By day 5 postinjection, the spleens of mice receiving 92 kBq of ^{225}Ac-CHX-DTPA weighed less than 15% of those of control mice. The slight increase in mean splenic weight at 4 h postinjection likely reflected an accumulation of both recently demarginated and dead leukocytes within the splenic parenchyma. Although not evaluated, a regenerative response was expected in these mice, because splenic and bone marrow recovery was noted on histopathologic evaluation at later time points.

The decreased WBC counts in mice receiving "cold" CHX-DTPA in MES was associated with no other adverse clinical or pathologic signs that would support a toxic effect of this mixture.

Morbidity and Histopathologic Damage Attributed to ^{225}Ac-CHX-DTPA

Mice receiving ^{225}Ac-CHX-DTPA were observed for signs of clinical illness as described above and were killed when indicated.

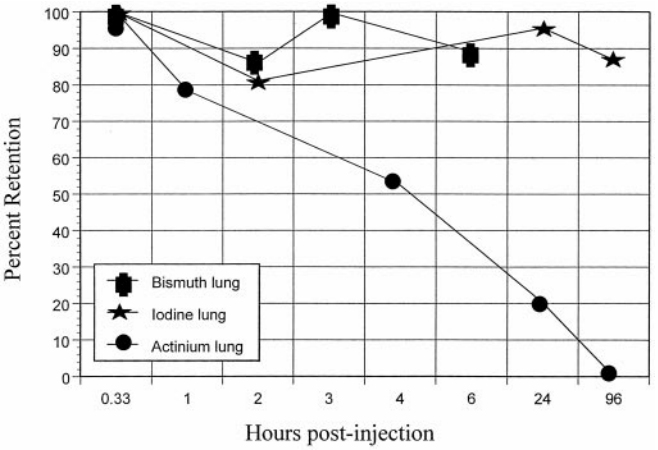

FIG. 4. Retention of radiolabeled monoclonal antibody 201B within the lung. Mice were injected with equivalent amounts of ^{225}Ac-CHX-DTPA Mab 201B (●), ^{125}I-201B (★), or ^{213}Bi-201B (✚), and sacrificed at indicated time points.

Tissue samples were collected and examined for histopathologic evidence of radiation toxicity. The organs showing the greatest evidence of radiation-induced toxicity included the bone marrow, spleen, GI tract, and the liver. Tissue damage was graded as mild, moderate, or severe based on cellular and whole organ changes and on the potential for recovery (Table 3).

By far the most dramatic changes in all organs, which correlated directly with the administered dose of ^{225}Ac-CHX-DTPA, appeared to involve cells of high metabolic activity or with high turnover rates. As early as 1 day postinjection, loss of cellularity was evident in both the bone marrow and splenic sections of mice receiving ^{225}Ac-CHX-DTPA at all doses and bone marrow cellularity was nearly obliterated by 5 days postinjection, with only an occasional normal cell seen in mice receiving 185, 370, and 740 kBq ^{225}Ac-CHX-DTPA. However, islands of normal hematopoietic precursors were retained in mice receiving 92 kBq ^{225}Ac-CHX-DTPA at this same time point and, although greatly reduced in number, up to day 14 days postinjection. This same group of mice, while exhibiting an estimated >50% reduction in total splenic leukocyte numbers, maintained foci of extramedullary hematopoiesis (EMH) at all time points. By day 53 postinjection, the splenic red pulp was dominated by foci of EMH and the bone marrow cavities contained multiple foci of normal hematopoietic precursor cells in this group of mice. In contrast, the bone marrow of control mice was highly cellular, with evidence of hematopoiesis throughout the study period, and maintained a myeloid:erythroid precursor ratio of approximately 2:1. Additionally, the spleens of control mice were characterized by well-developed follicles within the white pulp and multiple sites of EMH along the splenic cords.

Examination of liver, GI tract, and renal sections from mice receiving 92 kBq ^{225}Ac-CHX-DTPA again revealed only mild pathology by day 5 postinjection. By day 14 postinjection, these changes were resolving and by day 53 postinjection, the tissue architecture was similar to that of control mice. In contrast, mice receiving higher doses of ^{225}Ac-CHX-DTPA had tissue damage that ranged from moderate at 185 kBq to severe at higher doses, visible as early as 1 day postinjection.

Hepatocyte damage was most prominent around central veins, whereas renal damage involved primarily the proximal tubules. Interestingly, the predominant damage to the GI tract involved

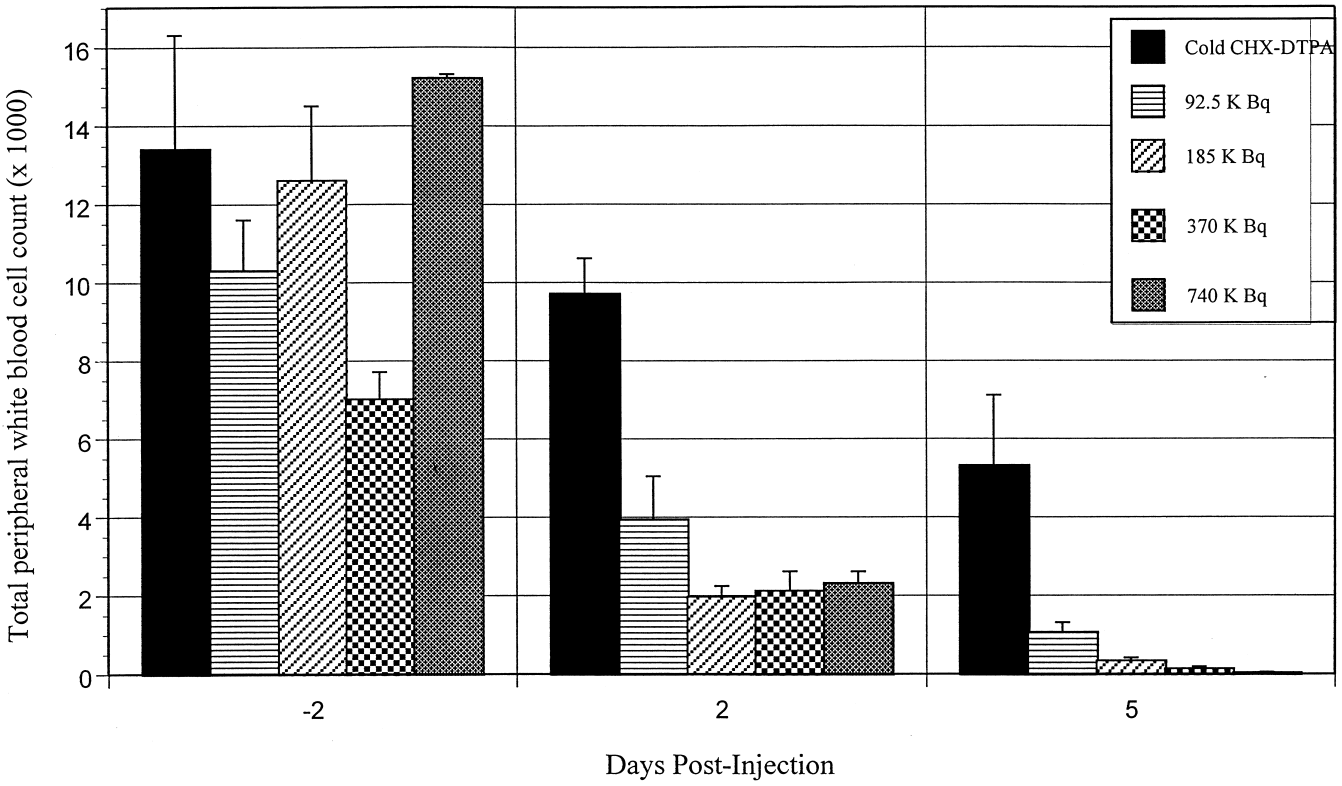

FIG. 5. Correlation of administered dose of ^{225}Ac-CHX-DTPA to total peripheral white blood cell counts (WBC). Adult BALB/c mice were injected intravenously with cold CHX-DTPA or varying doses of ^{225}Ac-CHX-DTPA and peripheral whole blood collected to determine WBC at the indicated time points. Values represent total WBC × 10^3 ± SD.

crypt cell precursors in the proliferation zone of the jejunal mucosa and gastric glandular cells.

In summary, ^{225}Ac-CHX-DTPA administered at 92 kBq caused severe but nonlethal bone marrow suppression, mild GI compromise, and mild cellular damage to the liver and kidney. However, when administered at greater than 92 kBq, tissue damage became more severe, involving multiple organs and lead to death.

DISCUSSION

Reports describing the biological behavior of ^{225}Ac are limited. Available data show that, like other actinides, intravenously injected, nonchelated ^{225}Ac distributes primarily to the liver and skeleton with some retention in the kidney (3, 6, 17, 24). The data presented in this study documented the organ distribution of various ^{225}Ac chelation complexes, determined the MTD of ^{225}Ac-CHX-DTPA, and described the tissue pathology associated with various doses of ^{225}Ac-CHX-DTPA in BALB/c mice. The data showed that the organ accumulation of ^{225}Ac was greatly reduced when chelated to EDTA and even further reduced when chelated to either CHX-DTPA or PEPA. When ^{225}Ac-CHX-DTPA was injected intravenously, the MTD was less than 185 kBq, because mice receiving this or higher doses demonstrated 100% mortality by day 8 postinjection. Mice receiving 92 kBq displayed no clinical signs of illness but had histopathologic evidence of radiation-induced organ toxicity that resolved over time.

TABLE 2. Mean Splenic Weights Following Administration of 225-Ac-CHX-DTPA

Time PI	Organ weight (g)
1 h	0.1018 ± 0.02
4 h	0.1263 ± 0.13
24 h	0.0671 ± 0.05
5 days	0.0152 ± 0.01

PI = postinjection.

Toxicity demonstrated in this study by modest amounts of ^{225}Ac has established the necessity of a chemically stable ligand, yet the ideal compound has not yet been identified. In an earlier study, intraperitoneal injection of ^{225}Ac in mice as an isotonic sodium citrate complex revealed predominant liver and bone distribution but, surprisingly, relatively high uptake by transplanted tumors (3). The high uptake by the liver was attributed to complex instability and the replacement of citrate as a ligand with nitrilotriacetic acid lead to significant increases in renal excretion, blood clearance, and tumor uptake, as well as decreases in liver accumulation (22). In our studies, chelation of ^{225}Ac with CHX-DTPA failed to prevent release of free ^{225}Ac to the liver and other organs, as evidenced by distribution and dosimetry data. PEPA provided little improvement over CHX-DTPA, but both ligands greatly reduced tissue accumulations of ^{225}Ac when compared with either acetate or EDTA complexes. It is estimated, based on these data, that PEPA and CHX-DTPA provide an approximate 75% improvement in ^{225}Ac chelation over EDTA.

The assessment of potential chelating ligands is preliminary to using the chelator compounds with protein targeting molecules. This approach has been demonstrated using CHX-DTPA as a

TABLE 3. Correlation of Tissue Pathology and 225-Ac Dose by Day 5 Postinjection

	Amount of injected actinium (kBq)			
	92	**185**	**370**	**740**
WBC count	3	3 +	3 +	3 +
Spleen	3	4	4	4
Bone marrow	3	4	4	4
Liver	2	3 +	4	4
GI tract	1	4	4 +	4 +
Kidney	2	4	4	4
Lung	0	1	1	1

WBC = white blood cell; GI = gastrointestinal.
Scale: 0 = no pathology, 1 = mild cellular swelling, 2 = cellular swelling with vacuolization, 3 = loss of cellular numbers, integrity, orientation, or structure, 4 = evidence of cellular necrosis.

chelating ligand. The formation of an ^{225}Ac-CHX-DTPA-murine anti-CEA IgG_1 prepared in acetate solution and injected intravenously to nude mice bearing colorectal tumors proved to be unstable *in vivo*; however, the study provided encouraging data on the preparation and potential use of radioimmunoconjugates (4). Our experience with ^{213}Bi-CHX-DTPA-Mab 201B provides strong evidence for the usefulness and efficiency of directed radioimmunotherapy (11, 13, 14). In the limited data presented here, ^{225}Ac-CHX-DTPA-Mab 201B appeared to retain similar bioactivity. Unfortunately, the biological half-life of this complex is short (~4 h), much shorter than the 10-day physical half-life of ^{225}Ac, thereby allowing for accumulation of uncoupled ^{225}Ac to various tissues. Thus, for ^{225}Ac targeting purposes, more stable conjugates must be found.

The acute decrease in total peripheral WBC counts following the intravenous administration of ^{225}Ac-CHX-DTPA was highly correlated with the injected dose. However, a portion of this decrease in WBC counts may be due to an as yet undetermined effect of either CHX-DTPA or the injection buffer, MES. This effect may explain the decrease in WBC counts seen in control mice. Although peripheral WBC counts vary widely in mice (9) ($6–15 \times 10^3$ cells/mm^3), the WBC counts in control mice from this study decreased to just below this lower limit by day 5 postinjection (Fig. 5). A dilutional effect on the WBC counts is possible given that the injection volume of 200 μL is approximately 1/8 the total blood volume of a normal mouse (9). However, this effect would have been short lived and corrected physiologically much earlier than 5 days. Organ histopathologic changes do not support a toxic effect of CHX-DTPA in MES, because no damage was seen in tissues of control mice throughout the experimental periods. Thus, although the changes in WBC counts in control mice are as yet unexplained, this response did not appear to have any lasting adverse effects on this group of mice. In retrospect, following WBC values throughout the experimental period would have provided useful information.

The tissue pathology observed in organs from mice receiving ^{225}Ac-CHX-DTPA (Table 3) correlated well with absorbed doses of ^{225}Ac (Table 1) and reflected both the injected dose (Fig. 1) and the instability of the complex. At the injected dose of 92 kBq ^{225}Ac-CHX-DTPA, absorbed doses of 30 Gy produced repairable damage to the liver. Absorbed doses of 8 Gy to the bone marrow and 4.5 Gy to the spleen caused near total ablation of lymphoid cells from these organs and from the circulation. These values are lower than the thresholds for normal tissue damage; however, two points may be made here: first, hematopoietic cells may be highly sensitive to α-particle irradiation and thus show acute and severe damage. Second, this effect was transient at the injected dose of 92 kBq, because both bone marrow and splenic hematopoietic cellularity was restored by 53 days postinjection. Although the dosimetry model (25) does not distinguish the different sections of the GI tract, the stomach and large intestine showed extensive nonrepairable damage with an absorbed dose of about 8 Gy (data not shown).

The toxic effects of ^{225}Ac-CHX-DTPA, as discussed above, were noted predominantly in hematopoietic tissues, liver, GI tract, and kidney. At an administered dose of 92 kBq, there was evidence of complete resolution of damage in all tissues examined by day 53 postinjection, based on histopathologic evaluation. At doses of ≥185 kBq, the cumulative tissue damage and immunosuppressive effects are presumed to contribute to death.

The potential toxic effects of the daughter decay products of ^{225}Ac can only be speculated for mice. A list of emissions from the decay of ^{225}Ac and daughters is shown in Table 4 (7, 23). Specifically, ^{213}Bi has not been shown to be toxic at equivalent doses administered in this report (14) and the distribution of ^{221}Fr, which has a $t_{½}$ of just 4.8 min, is unknown (18). Thus, for the purposes of this study, we attributed the toxicity observed following intravenous administration of ^{225}Ac-CHX-DTPA to the instability of the chelator and resultant accumulation of "free" radionuclide to organs and the damage caused therein to ^{225}Ac decay and not to decay of redistributing daughter radioisotopes. In aqueous solutions,

TABLE 4. Decay Properties of 225-Actinium

Radionuclide	Half-life	Decay mode (%)[a]	Decay product	Av.E_α or E_β[b] (keV)	$E_\gamma(I_\gamma)$ (keV)(%)[c]
^{225}Ac	10 days	α	^{221}Fr	5,750	188 (0.46)
^{221}Fr	4.8 min	α	^{217}At	6,357	218 (10.9)
^{217}At	32 ms	α	^{213}Bi	7,065	—
^{213}Bi	45.6 min	β (97.8)	^{213}Po	444	440 (26)
		α (2.16)	^{209}Tl	5,869	—
^{213}Po	4.2 μs	α	^{209}Pb	8,375	—
^{209}Tl	2.2 min	β	^{209}Pb	659	117 (81)
					467 (81)
					566 (98)
^{209}Pb	3.3 h	β	^{213}Bi (stable)	198	—

[a] When not indicated, branching is 100%.
[b] $\langle E_\beta \rangle$ = (Av. E_β. $I_\beta/\sum I_\beta$, $\langle E_\alpha \rangle$ = E_α. $I_\alpha)/\sum I_\alpha$.
[c] Number of emissions per 100 decay.

and in the absence of any chelating agent, ^{225}Ac (similar to other actinides and rare earth elements) undergoes hydrolysis forming $[Ac(OH)_{3-x}]^{x-}$. Due to the sub-picomolar concentrations of ^{225}Ac, the hydroxide species of ^{225}Ac, in turn, form radiocolloids and bind to the surfaces of reaction vessels and impurities commonly present in solutions. In living systems, the forms of "free" or uncomplexed species of ^{225}Ac is more uncertain because albumin and transferrin in serum contain large numbers of metal binding sites and may complex ^{225}Ac to some degree. Although we can only speculate on the mechanism of the high liver and bone uptake of all investigated ^{225}Ac species, early accumulation (hours to days) is common for all rare earth elements, which include lanthanides and actinides. It is difficult to estimate the stability required of a radionuclide complex to prevent tissue damage by released radionuclide yet still provide sufficient activity to be therapeutically useful. However, based on the data presented here, which evaluated the damaging effects of increasing amounts of injected ^{225}Ac-CHX-DTPA, 92 kBq of ^{225}Ac caused repairable tissue damage but 185 kBq of ^{225}Ac was lethal. Considering the stability of the CHX-DTPA complex, one may estimate that an accumulation of about 50 kBq of free ^{225}Ac represents a lethal burden to the whole mouse. Extrapolating this amount to therapeutic doses (1–2 MBq), whether or not the radionuclide is directed to specific tissues by Mabs, a successful chelator of ^{225}Ac must possess at least 95% stability over the half-life of the radioisotope. CHX-DTPA provides inadequate chelation, particularly because the amount of ^{225}Ac retained when injected at therapeutic doses would likely be less than optimal and result in toxic accumulations of free ^{225}Ac in organs. The stability constants of ^{225}Ac with ligands used in our study have not been reported, with the exception of the K_{eq} for ^{225}Ac-EDTA, which has a value of 1.0×10^{14} (1). Further, limited work in our laboratory using DOTA as a chelating ligand ^{225}Ac, following established methods (20), proved similarly unrewarding. ^{225}Ac-DOTA complexed to Mab 201B resulted in <20% retention of ^{225}Ac as confirmed by HPLC. Clearly then, the development of more stable chelation complexes is necessary to realize the therapeutic potential of ^{225}Ac. Current work with macrocyclic ligands, similar to PEPA, is ongoing and aimed at providing successful directed radiotherapy with minimal tissue toxicity.

The data presented indicate that release of ^{225}Ac *in vivo* from four currently available chelators results primarily in bone and liver accumulation. Toxicity and damage to bone marrow, liver, and other organs is irreversible at injected ^{225}Ac doses of greater than 92 kBq. Therefore, whereas our data suggest that ^{225}Ac-CHX-DTPA is among the most effective of the radionuclide-chelator complexes evaluated with regard to *in vivo* stability, more stable chelators and the determination of doses that minimize toxicity to nontarget tissues are essential to the successful use of ^{225}Ac in therapeutic models.(7, 8, 23)

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