Aminopolycarboxylate Chelators Are Effective for Whole-body Clearance of Free ^{225}Ac : A Method to Reduce Unexpected Radiation Exposure During Targeted Alpha Therapy

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Abstract

Purpose: Actinium-225 (^{225}Ac) is a promising radionuclide used in targeted alpha therapy (TAT). Although ^{225}Ac labelling of bifunctional chelating ligands is effective, previous in vivo studies have reported that free ^{225}Ac can be released from the drugs. Notably, such free ^{225}Ac predominantly accumulates in the liver and can cause unexpected toxicity. To accelerate the clinical development of ^{225}Ac TAT, methods for addressing unexpected toxicity are therefore needed. In this study, we evaluated various chelators in vitro and in vivo with regard to reducing and excreting free ^{225}Ac and compared their chemical structures.

Methods: Nine candidate chelators (D-penicillamine, dimercaprol, Ca-DTPA, Ca-EDTA, CyDTA, GEDTA TTHA, Ca-TTHA, and DO3A) were tested. In vitro interaction of ^{225}Ac and chelators was investigated. Biodistribution and dosimetry of free ^{225}Ac were examined in mice prior to the in vivo chelating study. For in vivo chelation, nine candidate chelators were administered 1 h after free ^{225}Ac injection, and biodistribution was compared 4 h after ^{225}Ac injection in mice. Two favourable chelators were then investigated intensively for biodistribution 24 h after the ^{225}Ac injection.

Results: The liver exhibited pronounced ^{225}Ac uptake corresponding to an estimated human absorbed dose of $4.76 \text{ Sv}_{\text{RBE5}}/\text{MBq}$. Aminopolycarboxylate chelators with five and six carboxylic groups, Ca-DTPA and Ca-TTHA, significantly reduced ^{225}Ac retention in the liver (22% and 30%, respectively). Significant ^{225}Ac reductions were observed in the heart and the remainder of the body with both Ca-DTPA and Ca-TTHA, and in the lung, kidney, and spleen for Ca-TTHA. In vitro interaction analysis supported the in vivo reduction ability of Ca-DTPA and Ca-TTHA.

Conclusions. Aminopolycarboxylate chelators with five and six carboxylic groups, Ca-DTPA and Ca-TTHA, were effective for whole-body clearance of free ^{225}Ac , with a significant reduction in the liver. This method could reduce undesirable radiation exposure from free ^{225}Ac during ^{225}Ac TAT.

Introduction

Actinium-225 (^{225}Ac) is a promising α -particle-emitting radionuclide used in targeted alpha therapy (TAT) [1, 2]. ^{225}Ac (half-life = 9.92 d) generates short-lived daughter nuclides and emits four high-linear energy transfer α particles in total until a long half-life of ^{209}Bi (2.01×10^{21} years) is reached. This induces lethal damage to target cancer cells in ^{225}Ac TAT [1, 2]. High-quality ^{225}Ac is easily produced from ^{229}Th generators (half-life = 7.3 y). ^{225}Ac is compatible with various targeting agents, such as peptides, antibodies, and nanoparticles, and ^{225}Ac -labeled drugs exhibit great efficacy in vivo [1, 2]. ^{225}Ac -PSMA-617 is a promising agent for prostate cancer metastasis as reported in preliminary clinical studies [3] and is currently under phase 1 clinical trials (AcTION: NCT04597411). Various ^{225}Ac -labeled antibodies have also been developed in clinical trials, such as ^{225}Ac -FPI-1434 and ^{225}Ac -lintuzumab (NCT03867682) [4, 5].

Numerous bifunctional chelating ligands for ^{225}Ac labelling of drugs have been evaluated [6, 7]. DOTA is used as the most common bifunctional chelating ligand in ^{225}Ac -labeled drugs and have been transferred to recent clinical trials [6, 7]. Although such bifunctional chelating ligands are highly useful and effective for ^{225}Ac labelling, previous in vivo studies have also observed that free ^{225}Ac can be released from the TAT drugs and that such free ^{225}Ac is distributed predominantly in the liver, followed by the bones [1, 6–12]. A previous study reported that free ^{225}Ac administration caused morphological changes in mouse livers, such as diffuse hepatocellular cytoplasmic vacuolation, consistent with glycogen accumulation or hydropic degeneration [9]. The free ^{225}Ac administration did not cause morphological changes in the bones; i.e., the bone marrow contained cellular progenitors from myeloid, erythroid, megakaryocyte similar to the control, although a transient decrease in white blood cells was observed [9]. This might be because α particles emitted from radionuclides that accumulate on the bone surface have little effect on the bone marrow owing to the short range [13, 14]. Therefore, the liver is considered as the major critical organ for free ^{225}Ac . Accordingly, both the development of methods for reducing the unexpected toxicity caused by free ^{225}Ac as well as drug development are important to accelerate the clinical studies for ^{225}Ac TAT.

Several chelator drugs are clinically used to reduce toxicity caused by non-radioactive and radioactive heavy metals that are unexpectedly accumulated in the body. Nine candidate chelators were investigated in this study, including D-penicillamine, dimercaprol, Ca-DTPA, Ca-EDTA, CyDTA, GEDTA, TTHA, Ca-TTHA, and DO3A (the chemical names are shown in Table 1) (Fig. 1). Penicillamine is used to reduce copper accumulation in Wilson's disease [15]. Dimercaprol is indicated for the treatment of arsenic, gold, and mercury poisoning [16]. Ca-EDTA is indicated for treating acute lead poisoning [17]. Ca-DTPA is used for treating internal contamination with plutonium, americium, or curium [18]. We hypothesised that the aforementioned chelators could also reduce the free ^{225}Ac released from drugs during ^{225}Ac TAT and accumulated in the body. CyDTA, GEDTA, and TTHA are commercially available aminopolycarboxylate chelators that form a stable complex with the lanthanum (III) ion (La^{3+}), which has a similar chemical nature (3+ charge and closed subshell electron configuration) and close ionic radius to actinium (Ac^{3+}) [19–21]. Ca-TTHA was included in our study to avoid potential Ca depletion in the body by TTHA. DO3A is a macrocyclic chelator that forms a stable complex with gadolinium and is used as a contrast agent for magnetic resonance imaging in clinical practice [22]. DOTA was not used in this study as labelling of biomolecules with ^{225}Ac is conventionally performed using DOTA [23, 24]. Other macrocyclic aminopolycarboxylates were not considered from a kinetic viewpoint; e.g., cyclic chelators require a longer time than acyclic chelators to form thermodynamically stable complexes [25]. This kinetic inertness would be a disadvantage in a flow system such as the blood stream.

Table 1

Candidate chelators to reduce free actinium-225 (^{225}Ac) (Dose, route, information)

Chelators	Abbreviations	Provider	Dose	Administration volume and route
D-Penicillamine	-	Fujifilm Wako Chemicals	300 mg/kg	150 μL p.o.
2,3-Dimercapto-1-propanol	Dimercaprol	Tokyo Chemical Industries	60 mg/kg	50 μL i.m.
Calcium diethylenetriamine- <i>N,N,N,N',N'</i> -pentaacetate	Ca-DTPA	Chemisch-pharmazeutische Fabrik GmbH	150 mg/kg	100 μL i.p.
Calcium ethylenediamine- <i>N,N,N,N</i> -tetraacetate	Ca-EDTA	Fujifilm Wako Chemicals	150 mg/kg	100 μL i.p.
<i>trans</i> -1,2-Diaminocyclohexane- <i>N,N,N,N</i> -tetraacetic acid	CyDTA	Fujifilm Wako Chemicals	150 mg/kg	100 μL i.p.
<i>O,O'</i> -Bis(2-aminoethyl)ethyleneglycol- <i>N,N,N,N</i> -tetraacetic acid	GEDTA	Fujifilm Wako Chemicals	150 mg/kg	100 μL i.p.
Triethylenetetramine- <i>N,N,N,N',N'',N''</i> -hexaacetic acid	TTHA	Fujifilm Wako Chemicals	150 mg/kg	100 μL i.p.
Calcium triethylenetetramine- <i>N,N,N,N',N'',N''</i> -hexaacetate	Ca-TTHA	See text	150 mg/kg	100 μL i.p.
1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid	DO3A	Macrocyclics	150 mg/kg	100 μL i.p.

In this study, we examined the interaction and effects of the nine chelators with ^{225}Ac in vitro and on the biodistribution of free ^{225}Ac in mice. We also evaluated the association between reduction in ^{225}Ac retention and the chemical structure of the chelators.

Materials And Methods

Radionuclides

^{225}Ac ($T_{1/2} = 9.92$ d) was obtained from a ^{229}Th ($T_{1/2} = 7880$ y) stock solution provided by the Laboratory of Alpha-Ray Emitters, Institute for Materials Research, Tohoku University. In the ^{229}Th stock solution,

^{225}Ac had reached a sufficient equilibrium state with its parent nuclides, ^{229}Th and ^{225}Ra ($T_{1/2} = 14.9$ d). ^{225}Ac was separated from ^{229}Th using a previously reported method [26] with slight modifications. Briefly, ^{225}Ac was separated from the ^{229}Th solution (5 mL) prepared in 7M HNO_3 passing through a 5×42 mm column containing Muromac AG1 $\times$ 8 anion exchange resin (Muromachi Technos Co., Ltd., Tokyo, Japan) pre-equilibrated with 7 M HNO_3 . The column was washed with 15 mL of 7 M HNO_3 to collect trace amounts of ^{225}Ac . The eluate was diluted to 4M HNO_3 and purified by separation of ^{229}Th and ^{225}Ra using a tandem combination of UTEVA Resin and DGA Resin (Eichrom Technologies, LLC, Lisle, IL, USA). In this system, ^{225}Ra was passed through all cartridge systems, whereas trace amounts of ^{229}Th and the desired ^{225}Ac were retained by UTEVA Resin and DGA Resin, respectively. ^{225}Ac was then recovered from DGA Resin using 10 mL of 0.05 M HNO_3 . The eluted ^{225}Ac solution was evaporated nearly to dryness, then re-dissolved in 0.1M HCl as a stock solution. The ^{225}Ac solution was adjusted to neutral pH using 3 M ammonium acetate buffer (pH 6.0); the solution was then diluted to a concentration of 10 kBq/100 μL with saline for in vitro and in vivo studies. The radioactivity of ^{225}Ac was quantified using a germanium semiconductor detector (ORTEC, SEIKO EG&G, Tokyo, Japan). Following ^{225}Ac separation, ^{229}Th was recovered from UTEVA Resin with 0.5 M HCl for ^{225}Ac ingrowth.

Preparation of reagents

The chelators used in this study are listed in Table 1. Ca-DTPA and D-penicillamine were dissolved in sterile water. Ca-EDTA, GEDTA, CyDTA, TTHA, and DO3A were dissolved in sterile water with an aliquot of sodium hydroxide, and the pH was adjusted between 8–9, if necessary. Ca-TTHA was prepared by adding an equal amount of calcium dichloride to TTHA in sterile water, and the pH was adjusted between 8–9 using a sodium hydroxide solution. Dimercaprol was diluted with peanut oil (Nacalai Tesque, Kyoto, Japan).

In vitro analysis of ^{225}Ac chelate formation

^{225}Ac solution (20 mL; 10 kBq/100 μL) was mixed with 20 μL of chelator solution (Table 1) and incubated at 37°C for 30 min. A 1 mL aliquot of each sample was applied onto the chromatography papers (3MM, Whatman, Little Chalfont, UK) and developed with 0.9 % NaCl as the mobile phase. This system is reported to separate polar ^{225}Ac -chelator complexes from unbound ^{225}Ac , and the ^{225}Ac chelate was observed at the solvent front and free ^{225}Ac was observed at the origin [27]. Radioactivity was analysed on the TLC plates using a bioimaging analyser (FLA-7000, GE Healthcare, Chicago, IL, USA).

Animals

BALB/cAnNCrCrj female mice (6 weeks old, 18–22 g body weight) were obtained from Charles River Laboratories. The mice were weighed and randomised for analysis after more than seven days of acclimatisation. All animal experiments were approved by the Animal Ethics Committee of our institution and conducted in accordance with institutional guidelines.

Biodistribution of free ^{225}Ac in mice and dosimetry analysis

Biodistribution was studied in four mice per group at 5 min, 1 h, 4 h, 24 h, and 72 h after the injection with free ^{225}Ac . Animals in the 5 min to 4 h sampling groups were housed individually, and the urine and faeces were collected with polyethylene-laminated filter papers. Animals in the 24 h and 72 h sampling groups were housed individually in metabolic cages (3600M021; Tecniplast S.p.A, Buguggiate, Italy) to collect urine and faeces. Organs of the mice, such as the salivary glands, heart, lung, liver, kidney, spleen, pancreas, brain, muscle, and femur, along with the remainder of the body and the blood were collected and weighed. Radioactivity levels were measured using a γ -counter (2480 Wizard 2; PerkinElmer, Waltham, MA, USA). The biodistribution data were calculated and are shown as the % of injected dose (ID)/g for the organs and blood and the % ID for the urine and faeces. The mean absorbed doses of ^{225}Ac (mSv/MBq) in humans were estimated based on biodistribution data. The mean % ID/g values of the mouse organs were converted into the corresponding human values [28]. These values were processed with the OLINDA/EXM version 1.1 software [29], which used the relative biological efficacy (RBE) for α particles of 5 and a dynamic bladder model with a voiding interval of 4.8 h to estimate the organ doses (Sv RBE_5/MBq). Considering instant decay of the daughter nuclides of ^{225}Ac (^{221}Fr , ^{217}At , ^{213}Bi , ^{213}Po , ^{209}Tl and ^{209}Pb) without translocation during the decay, the residency times of ^{225}Ac were forwarded as those of daughter nuclides [3].

In vivo chelation study

The schedule of the in vivo chelation study is summarised in Supplementary Material Figure S1.

Experiment 1

^{225}Ac (10 kBq/mouse) was intravenously injected into the mice. At 1 h post ^{225}Ac injection, the mice were treated with each chelator solution, except for the untreated controls. A total of five mice were used in each chelator solution group, except for the Ca-TTHA group, which contained four mice. The administered doses and routes are summarised in Table 1 and were determined as follows: Dimercaprol was administered intramuscularly (i.m.) to the mice, and the dose was approximately half of the LD_{50} in mice, or 125 mg/kg [30]; D-penicillamine was administered orally (p.o.) to the mice, and the dose was determined according to our previous study with ^{64}Cu -ATSM and D-penicillamine [31]. Ca-DTPA and the remaining compounds were intraperitoneally (i.p.) administered, and their doses were sufficiently lower than the LD_{50} of Ca-DTPA in mice (6,216.9 mg/kg) [32]. The mice were housed individually, and urine and faeces were collected using polyethylene-laminated filter papers. At 4 h after ^{225}Ac administration, the animals were euthanised, and biodistributions were evaluated as described above.

Experiment 2

Ca-DTPA and Ca-TTHA showed the highest reduction in the rate of liver uptake among the chelators tested; therefore, their effects were observed over a longer time period. At 1 h following ^{225}Ac injection (10 kBq/mouse), the mice were administered solutions of Ca-DTPA or Ca-TTHA, except for the untreated controls. A total of four animals were included in each chelator solution group. At 24 h following ^{225}Ac administration, the mice were euthanised and the free ^{225}Ac biodistributions were evaluated as described

above, as 24 h is the peak time point of biodistribution observation for the liver. The time-activity curves were created using the biodistribution data at 4 h and 24 h to compare the effects of these chelators to those of the control.

Statistical analysis

Data are expressed as the means $\pm$ SD. Multiple comparisons were conducted using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test with post hoc comparisons using Tukey–Kramer or Steel–Dwass tests. Time activity curves were compared using a two-way repeated ANOVA. Data analyses were conducted using JMP 13.2.0 software (SAS Institute). $P < 0.05$ was considered to be statistically significant.

Results

In vitro analysis of ^{225}Ac chelate formation

The ability of the chelators to capture ^{225}Ac in vitro is shown in Fig. 2. Free ^{225}Ac (control) remained at its origin. In contrast, the ^{225}Ac with Ca-DTPA, Ca-EDTA, Ca-TTHA, and TTHA developed sharply, with more than 95% of the radioactivity at the solvent front. D-penicillamine and dimercaprol exhibited a weak ability to move ^{225}Ac from its origin, and most of the radioactivity remained at the origin. For the remaining chelators, 30–70% of radioactivity was found between the origin and front of the chromatogram.

Biodistribution and dosimetry of ^{225}Ac in mice

Time-activity curves for the collected organs and urinary and faecal excretions are shown in Fig. 3. Noticeable ^{225}Ac accumulation was observed in the liver followed by the bones throughout the observation period. A small amount of ^{225}Ac , less than 5 % of the total dose at 72 h after administration, was excreted in the faeces. Additionally, a negligible amount of ^{225}Ac was excreted through the urine in mice. These results show a low whole-body clearance of free ^{225}Ac in mice. The mean absorbed doses of ^{225}Ac (mSv/MBq) in humans were estimated based on the biodistribution data from mice (Table 2). The liver and bone showed relatively high human estimated absorbed doses.

Table 2

Mean estimated human absorbed doses for free actinium-225 (^{225}Ac),
extrapolated from mouse biodistribution data

Estimated absorbed dose (mSv _{RBE5} /MBq)		
Target Organ	Male	Female
Adrenals	2.34E + 02	3.03E + 02
Brain	5.62E + 00	6.66E + 00
Breasts	2.34E + 02	3.03E + 02
Gallbladder wall	2.34E + 02	3.03E + 02
Lower large intestinal wall	2.34E + 02	3.03E + 02
Small intestine	2.34E + 02	3.03E + 02
Stomach wall	2.34E + 02	3.03E + 02
Upper large intestinal wall	2.34E + 02	3.03E + 02
Heart wall	3.85E + 02	5.06E + 02
Kidneys	2.15E + 02	2.33E + 02
Liver	4.76E + 03	6.50E + 03
Lungs	1.61E + 02	2.01E + 02
Muscle	2.86E + 01	4.70E + 01
Ovaries	2.34E + 02	3.03E + 02
Pancreas	4.79E + 01	5.33E + 01
Red marrow	3.37E + 02	3.89E + 02
Osteogenic cells	1.17E + 04	1.63E + 04
Skin	2.34E + 02	3.03E + 02
Spleen	3.79E + 02	4.62E + 02
Testes	2.34E + 02	
Thymus	2.34E + 02	3.03E + 02
Thyroid	2.34E + 02	3.03E + 02
Urinary bladder wall	2.36E + 02	3.05E + 02
Uterus	2.34E + 02	3.03E + 02
Total body	4.45E + 02	5.78E + 02

	Estimated absorbed dose (mSv _{RBE5} /MBq)	
Effective dose equivalent	8.72E + 02	1.17E + 03
Effective dose	5.64E + 02	7.52E + 02

Effect of chelator administration in vivo

Experiment 1

Figure 4 shows differences in the biodistribution of ^{225}Ac activities at 4 h following ^{225}Ac injection between the control and chelator groups. Ca-DTPA, Ca-EDTA, GEDTA, TTHA, and Ca-TTHA showed significant reductions in the liver uptake of ^{225}Ac ($P < 0.05$). There were significant reductions in ^{225}Ac with Ca-DTPA, GEDTA, TTHA, and Ca-TTHA in the heart, and with Ca-DTPA, TTHA, and Ca-TTHA in the remainder of the body ($P < 0.05$). Urinary excretion of ^{225}Ac was significantly accelerated with Ca-DTPA, TTHA, and Ca-TTHA ($P < 0.05$). There was a moderate positive correlation between the levels of chelator interactions with free ^{225}Ac in vitro and ^{225}Ac reduction in the liver in vivo on scatter plot analysis ($R^2 = 0.526$, $P < 0.05$) (Supplementary Material Figure S2).

Experiment 2

Ca-DTPA and Ca-TTHA were selected among the other chelators to observe their effects over a longer period as they showed a higher reduction rate of ^{225}Ac liver uptake and urine excretion. At 1 h post ^{225}Ac administration, Ca-DTPA and Ca-TTHA were administered to the mice similarly as performed in Experiment 1, and the mice were euthanised 24 h following ^{225}Ac injection. Figure 5 and Supplementary Material Figure S3 show the time-activity curves of the selected organs of the control and chelator groups. The time activity curve analysis showed that the liver, heart, and remainder of the body exhibited significant ^{225}Ac reductions in the Ca-DTPA and Ca-TTHA groups, and the lung, kidney, spleen, and pancreas showed a significant ^{225}Ac reduction in the Ca-TTHA group ($P < 0.05$, vs. control). The reduction in the area under the curve of the liver was 22% with Ca-DTPA and 30% with Ca-TTHA (Fig. 5 and Supplementary Material Figure S3). For the femur, ^{225}Ac retention was reduced although not significantly. Ca-DTPA and Ca-TTHA showed significant increases in urinary excretion of ^{225}Ac , and Ca-TTHA showed a significant increase in the faecal excretion of ^{225}Ac ($P < 0.05$ vs. control), but Ca-DTPA did not exhibit any significant increase in ^{225}Ac excretion.

Discussion

In this study, we demonstrated that aminopolycarboxylate chelators with five and six carboxylic groups, Ca-DTPA and Ca-TTHA, respectively, induced whole-body clearance of free ^{225}Ac with a significant reduction in the liver as the critical organ. ^{225}Ac was excreted in the urine and faeces after chelation. The liver showed the highest retention of free ^{225}Ac with an estimated human absorbed dose of 4.76

Sv_{RBE5}/MBq . Therefore, Ca-DTPA and Ca-TTHA administration may be a treatment option for unexpected radiation exposure caused by free ^{225}Ac .

Aminopolycarboxylate chelators, such as DTPA, EDTA, GEDTA, and TTHA, showed higher reductions in ^{225}Ac retention in the liver than those with D-penicillamine, dimercaprol, and DO3A. These results reflect a difference in the number of ligating atoms and the number of coordinating groups. The Ac^{3+} ion is classified as a 'hard' Lewis acid according to the hard and soft acids and bases (HSAB) theory [33], as it behaves similar to the La^{3+} ion, carrying a large charge and low polarisability. Thus, the Ac^{3+} ion prefers hard bases or non-polarisable and negatively charged Lewis bases such as carboxylates [34] and thus shows a high affinity for aminopolycarboxylates, especially those that have a higher number of carboxylic groups, such as DTPA and TTHA. D-penicillamine and dimercaprol are sulphur-coordinating soft Lewis bases that are effective decorporation chelators for soft metal ions, such as Pb^{2+} , Hg^{2+} , and As^{3+} .

The aminopolycarboxylates used in this study behave as multidentate chelators for the Ac^{3+} ion. The thermodynamic stability increases with the number of coordinating atoms [20]. Indeed, our results are consistent with the stability constants for La^{3+} complexes [35]. The low decorporation ability of DO3A can be explained in a similar manner. The low ability of CyDTA may occur because of kinetic issues, as considerable time is required to complete the coordination to the Ac^{3+} ion, thereby explaining why this chelator showed lower reduction.

Ca-TTHA and the sodium salt of TTHA exhibited similar effects on the ^{225}Ac distribution pattern in mice. Previous studies using EDTA have reported that its calcium salt, rather than the sodium salt, is preferred as a chelator drug because Na-EDTA chelates Ca in the body and may cause hypocalcaemic tetany [36]. Therefore, Ca-TTHA should be selected for the development of TTHA as a chelator drug.

In this study, we administered a series of chelators 1 h following ^{225}Ac injection. The chelator administration time was selected based on the distribution data indicating that the majority of the free ^{225}Ac was distributed in the liver. We found that Ca-DTPA and Ca-TTHA reduced free ^{225}Ac retention in the liver as well as in other organs. These results show that Ca-DTPA and Ca-TTHA are effective in reducing radiation exposure from free ^{225}Ac . Ca-TTHA showed a higher tendency to reduce ^{225}Ac retention compared to that by Ca-DTPA in the organs. These data support the development of Ca-TTHA for the removal of free ^{225}Ac . To produce ^{225}Ac for medical use, ^{229}Th generators are currently used, while accelerator synthesis of ^{225}Ac is under investigation to increase the supply [37]. In the accelerator synthesis, ^{227}Ac (half-life = 21.8 y), which cannot be chemically separated, is included as an unavoidable by-product [37]. The method proposed by the present study may thus also be useful to reduce the ^{227}Ac , which will be retained in the liver substantially longer than ^{225}Ac in ^{225}Ac TAT. Aminopolycarboxylates are known to form stable chelates with a wide range of metal ions. DTPA and TTHA can be expected to capture the daughter nuclide ^{213}Bi (and to a lesser extent, $^{209}Pb^{2+}$) de-chelated by the recoil during the decay process of ^{225}Ac , as these two chelators form highly stable complexes with Bi^{3+} [35].

This study had several limitations. First, the free ^{225}Ac was evaluated in non-tumour-bearing mice. Further preclinical and clinical studies with specific target ^{225}Ac -labeled drugs are necessary to determine the timing of administration not to avoid tumour uptake of ^{225}Ac in tumour-bearing mice. The ^{225}Ac internalisation by tumour cells following drug delivery is important in drug design in the development of agents for ^{225}Ac TAT, because internalisation causes short-lived daughter radionuclides generated by ^{225}Ac to be trapped in the cells [38, 39]. Therefore, the chelator administration timing would be appropriate after tumour delivery and internalisation, because chelators with negatively charged carboxylic groups do not penetrate tumour cell membranes [40]. Second, this study used a fixed administration dose and the same administration route for aminopolycarboxylate chelators (150 mg/kg, i.p.) for comparisons. Optimisation of the administration dose, route, and safety tests are necessary in future studies.

In conclusion, we found that aminopolycarboxylate chelators with five and six carboxyl groups, i.e. Ca-DTPA and Ca-TTHA, were useful for whole-body clearance of free ^{225}Ac , with marked reduction in the liver. Our findings provide a novel strategy to remove accumulated free ^{225}Ac released from ^{225}Ac -labeled drugs and encourage the future development of ^{225}Ac TAT.

Declarations

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Conflict of interest The authors declare no conflicts of interest.

Data availability Data available on request.

Code availability Not applicable

Authors' contributions M.Y., Y.Y., H.M., K.W. designed the study. M.Y., Y.Y., H.M., M.S., M.T., C.I., F.H., T.T., A.D., T.H., H.F., and K.W. performed the experiments and wrote the manuscript. All authors also made significant contributions by discussing the results and enhancing the manuscript for intellectual content. All authors approved the final version submitted.

Ethics approval All animal experiments were approved by the Animal Ethics Committee of our institution and conducted in accordance with institutional guidelines.

Consent to participate Not applicable

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Figures

Figure 1

Chemical structure of the candidate chelators. Possible ligating atoms are coloured.

Figure 2

In vitro ability of the chelators to capture actinium-225 (^{225}Ac). Paper chromatography of free ^{225}Ac alone (control) and free ^{225}Ac with chelators. The percentages of radioactivity at the solvent front (^{225}Ac chelates) are shown for each chromatograph. ND = not detected.

Figure 3

Biodistribution of free actinium-225 (^{225}Ac) in BALB/c mice. Data were obtained at 5 min, 1 h, 4 h, 24 h, and 72 h after intravenous ^{225}Ac injection. Values are expressed as % ID/g for the organs and blood and as the % ID for urine and faeces. Values are shown as the mean $\pm$ SD (n = 4).

Figure 4

Effect of chelator administration on the biodistribution of free actinium-225 (^{225}Ac). Biodistribution of ^{225}Ac in the control and chelator groups. Mice were euthanised 4 h after the ^{225}Ac injection. Values are shown as means $\pm$ SD; $n = 4-5$ (see details in the Methods section). * indicates statistical significance ($P < 0.05$, vs. control).

Figure 5

Time-activity curves of free actinium-225 (225Ac) after Ca-DTPA and Ca-TTHA administration in major organs, urine, and faeces. Time-activity curves were generated using the biodistribution of 225Ac in control mice and mice with Ca-DTPA and Ca-TTHA at 4 and 24 h following 225Ac injection. Values are shown as the means $\pm$ SD; n = 4–5. Numbers in the graphs show % increase (positive) or decrease (negative) of the area-under-the-curve in each chelator group, compared to the control. § indicates

statistical significance of time-activity curves ($P < 0.05$, vs. control). NS = not significant. * Indicates statistical significance at each time point ($P < 0.05$, vs. control). The data for the other organs are shown in Supplementary Material Figure S3.

Supplementary Files

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