From early prophylaxis to delayed treatment: Establishing the plutonium decorporation activity window of hydroxypyridinonate chelating agents

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Abstract

The potential consequences of a major radiological event are not only large-scale external radiation exposure of the population, but also uncontrolled dissemination of, and internal contamination with, radionuclides. When planning an emergency response to radiological and nuclear incidents, one must consider the need for not only post-exposure treatment for contaminated individuals, but also prophylactic measures to protect the workforce facing contaminated areas and patients in the aftermath of such events. In addition to meeting the desired criteria for post-exposure treatments such as safety, ease of administration, and broad-spectrum efficacy against multiple radionuclides and levels of challenge, ideal prophylactic countermeasures must include rapid onset; induce minimal to no performance-decrementing side effects; be compatible with current military Chemical, Biological, Radiological, Nuclear, and Explosive countermeasures; and require minimal logistical burdens. Hydroxypyridinone-based actinide decorporation agents have shown the most promise as decorporation strategies for various radionuclides of concern, including the actinides plutonium and americium. The studies presented here probe the extent of plutonium decorporation efficacy for two chelating agents, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), from early pre-exposure time points to a delay of up to 7 days in treatment administration, i.e., well beyond the first hours of emergency response. Despite delayed treatment after a contamination event, both ligands clearly enhanced plutonium elimination through the investigated 7-day post-treatment period. In addition, a remarkable prophylactic efficacy was revealed for 3,4,3-LI(1,2-HOPO) with treatment as early as 48 hours before the plutonium challenge. This work provides new perspectives in the indication and use of experimental actinide decorporation treatments.

1. Introduction

Incidents such as the Fukushima Daiichi Nuclear Power Plant accident in 2011 and the leak of radioactive materials at the U.S. Department of Energy Waste Isolation Pilot Plant in 2014 are reminders of the continuous need for safeguards in a world that increasingly relies on nuclear technologies. From generating electricity to being misused as components in radiation dispersal devices or “dirty bombs,” radiological materials serve purposes that range from the benevolent to the malevolent and impact not only local nuclear power users, but also those halfway around the world [1]. Whether natural or manmade, accidental or deliberate, the possibility of radiological incidents from laboratories, industry, or terrorism that lead to the uncontrolled dissemination of radioactive contaminants highlights the importance of developing effective decorporation therapies as medical countermeasures [2].

Prompt decorporation is crucial for mitigating both immediate and future biological effects from radiological contamination. Adverse health effects include tissue damage and the development of various cancers, and are dependent upon factors such as the quantity of contaminants and duration of contamination [3, 4]. Internal contamination, i.e., the deposition of radionuclides in the body via routes that include ingestion, inhalation, and absorption through wounds, is especially dangerous since it may produce local, systemic, or a combination of radiation effects [5].

To enhance emergency preparedness in the United States in response to potential nuclear accidents and terrorist threats, the U.S. Food and Drug Administration (FDA) approved two forms of diethylenetriamine pentaacetic acid (DTPA) in 2004 -- calcium (Ca-DTPA) and zinc (Zn-DTPA) -- to expedite the excretion of plutonium, americium, and curium after internal contamination [6]. Although it is the first and only drug approved for treating internal

contamination with the aforementioned radioactive elements, DTPA's efficacy is limited to certain forms of these elements, routes of administration, and dosages. The drug's efficacy is hindered when isotopes are mixed with other materials; it needs to be administered either intravenously or via nebulized inhalation depending on the route of contamination; and it must be taken in excess, which may be a result of its low absorption in the gastrointestinal tract [7-9]. Experiments have also shown that Ca-DTPA does not chelate plutonium after the element's deposition in organs, explaining the necessity of administering treatment as soon as possible post-contamination [10]. Additionally, DTPA's side effects include the loss of essential metals such as zinc and magnesium from the body, further emphasizing the need for alternative decorporation therapy.

Addressing the limitations of Ca-DTPA and Zn-DTPA, an octadentate hydroxypyridinone-based chelator, 3,4,3-LI(1,2-HOPO), has shown efficacy with high potency and low toxicity through parenteral and oral routes of administration, preferred qualities in drug development. Studies have not only considered sex bias by examining efficacy in both male and female mice, but also elucidated the ability of 3,4,3-LI(1,2-HOPO) to, at physiological pH, form stable, excretable complexes with those radiological elements chelated by DTPA along with additional ones [11-13]. Its efficacy and safety have been proven in multiple animal models in order to meet criteria in the FDA's *Animal Efficacy Rule* and gain approval [14-16] since efficacy trials in human beings cannot be ethically conducted [17-19]. A promising candidate for treating internal radionuclide contamination, 3,4,3-LI(1,2-HOPO) received an investigational new drug (IND) designation from the FDA in August 2014 and is awaiting phase I clinical trials.

To complement previous studies that showcased the efficacy of 3,4,3-LI(1,2-HOPO) and that of a second actinide chelator in simultaneous development, 5-LIO(Me-3,2-HOPO), the experiments

described herein probe the potential of delayed and prophylactic treatment via intraperitoneal injection or oral administration for internal plutonium contamination. Realistically, treatment for the majority of the population following a radiological incident will not be accessible until after the first 24 hours of emergency response; likewise, prophylaxis is crucial for first-responders and the military. Ideal prophylaxis should provide broad-spectrum protection against multiple isotopes and levels of challenge. It must also be safe, efficacious, have a rapid onset, be easily administered, induce minimal to no performance-decrementing side effects, be compatible with current military Chemical, Biological, Radiological, and Nuclear (CBRN) countermeasures, and require minimal logistical burdens. Consequently, the current limitations of DTPA-based products and the lack of a viable drug for use prior to exposure to radiological material stress the significance and urgency of developing novel, efficacious decorporation therapies such as 3,4,3-LI(1,2-HOPO).

We present two sets of studies aimed at investigating the administration time window of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO). Enhanced plutonium elimination is noted as early as 48 hours prior to contamination for prophylactic intraperitoneal or oral treatment, and as late as 7 days post-challenge for delayed intraperitoneal treatment. To ensure consistency among these and previously reported studies, the chosen animal model was the young adult female Swiss-Webster mouse, and contamination with soluble ^{238}Pu -citrate was performed through a single intravenous injection.

2. Materials and Methods

2.1 Contaminant and Test Article Solutions

A stock solution of ^{238}Pu -nitrate in 4 M HNO_3 was purchased from Eckert and Ziegler Isotope Products (Valencia, CA, USA) and used to prepare injection solutions. Contamination doses consisted of 0.2 mL aliquots of solutions containing 20 nCi (0.75 kBq, 1.16 ng) of ^{238}Pu in 0.008 M sodium citrate and 0.14 M NaCl, pH 4. The test articles 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were prepared by Synthetech, Inc. (Albany, OR, USA) and Albany Molecular Research, Inc. (Albany, NY, USA), respectively, as described previously [17]. DTPA was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was formulated as Ca-DTPA using CaCO_3 and NaOH, similarly to the formerly available drug product commercialized by Hameln Pharmaceuticals gmbh (Hameln, Germany). Test article solutions were prepared such that the selected dosages (30 $\mu\text{mol/kg}$ for Ca-DTPA, 30 or 100 $\mu\text{mol/kg}$ for 3,4,3-LI(1,2-HOPO), and 100 or 200 $\mu\text{mol/kg}$ for 5-LIO(Me-3,2-HOPO)) were contained in 0.5 mL of 0.14 M NaCl, with the pH adjusted to 7.4-8.4 with 1 N NaOH. All solutions were filter-sterilized (0.22 μm) prior to administration. The concentration of each solution was verified by high-performance liquid chromatography, following modified published methods [20].

2.2 Animals and General Procedures

All procedures and protocols used in the described *in vivo* studies were reviewed and approved by the Institutional Animal Care and Use Committee of Lawrence Berkeley National Laboratory and were performed in AAALAC accredited facilities. The animals used were young adult (86 ± 6 days old for delayed treatment experiment and 90 ± 3 days old for prophylactic treatment experiment) female (30.7 ± 4.0 g for delayed treatment experiment and 30.8 ± 1.6 g for

prophylactic treatment experiment) Swiss-Webster mice (Simonsen Laboratories, Gilroy, CA, USA). Gross body and tissue compositions, plasma, extracellular fluid, and red cell volumes of the whole body, major tissues and organs of these mice (intact or bled 25 to 40% of their total blood volume) have been determined previously [21]. Mice were kept under a 12-hour light cycle with controlled temperature (18-22°C) and relative humidity (30-70%), and were given water and food *ad libitum*. Each group of mice was housed together in a plastic stock cage lined with a 0.5 cm layer of highly absorbent low-ash pelleted cellulose bedding (ALPHA-dri®) for separation of urine and feces. Intravenous (iv) injections into a warmed lateral tail vein, intraperitoneal (ip) injections, oral administrations (po, through gastric intubation) and euthanasia were performed under isoflurane anesthesia. To probe the effect of delayed treatment, groups of five mice were injected iv with a single dose of ^{238}Pu -citrate, and ligand or control saline solutions were administered ip once at the following post-contamination treatment times: 1 h, 5 h, 16 h, 24 h, 3 d, 7 d. Excreta were collected daily for 7 days. Animals were euthanized 7 days after treatment. To probe the effect of prophylactic treatment, groups of five mice were first administered ligand or control saline solutions ip or po once at the following pre-contamination treatment times: -1 h, -6 h, -16 h, -24 h, -30 h, -40 h, -48 h. Mice were then injected iv with a single dose of ^{238}Pu -citrate and excreta were collected daily for 3 days. Animals were euthanized 3 days (72 h) after contamination. Mice were euthanized by cervical dislocation over their respective cage to collect the urine expelled at death, and immediately wrapped in plastic and frozen for later dissection.

2.3 Tissue Sampling and Processing

After partial thawing of the frozen mice, livers and kidneys were dissected, and the abdominal tissue remainder (ATR, which includes intact gastrointestinal (GI) tract, reproductive organs,

spleen, urinary bladder, and abdominal fat) was removed. The livers, kidneys, ATR, and partially eviscerated carcasses were managed as individual samples. Feces samples were separated manually from urine-stained cellulose bedding and treated as group samples (one group per cage). All samples were dried at 100°C and dry ashed at 575°C. The ashed samples were treated with concentrated HNO₃. These acidified solutions were then homogenized in dilute HNO₃ and mixed with Ultima Gold (Perkin Elmer, Shelton, CT, USA) for detection of radiotracers by liquid scintillation counting (Packard Tri-Carb model B4430, Perkin Elmer).

2.4 Data Management and Analysis

All experiments used radioactive ²³⁸Pu as a tracer and were therefore managed as metabolic balance studies, in which all tissues and excreta were radioanalyzed; average radiochemical recoveries were all greater than 95% injected dose (ID). The experimental data are reported as radionuclide fractions, expressed as percent of injected ²³⁸Pu (%ID), and values are arithmetic means ± SD. When comparing values between groups in the decorporation studies, the term “significant” is used in the statistical sense, indicating $p < 0.01$ by one-way analysis of variance (ANOVA) followed by adequate post-hoc analysis. The Dunnett’s multiple-comparison test was used to compare groups of animals treated with a chelating agent to the corresponding control group that was administered saline, while the Tukey’s Honestly Significant Difference (HSD) multiple-comparison test was used to perform pairwise comparisons between all groups treated with a chelating agent. Both tests were set at the 99% confidence interval level. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

The plutonium elimination enhancement promoted by a single parenteral or oral administration with one of the experimental decorporation agents 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) was probed in young adult female Swiss-Webster mice, dependent on the time of treatment administration. In the first study, chelation treatment was administered parenterally once after the challenge event, at times varying from 1 hour to 7 days post-contamination, and mice were euthanized 7 days after treatment. In the second study, a single treatment was administered prophylactically either parenterally or orally at times varying from 48 hours to 1 hour prior to contamination, and mice were euthanized 3 days after the contamination event. In both studies, the primary efficacy endpoint was radionuclide body content reduction at the time of euthanasia, compared to the saline-treated control groups. For parenteral treatment cases, comparisons could also be drawn with DTPA-treated groups. The *in vivo* portions of both studies were accomplished without incident. As expected from previously reported results, dose levels for the experimental ligands 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) did not result in any discernable adverse effects.

3.1 Delayed Parenteral Chelation Treatment

For the study where mice were treated between 1 hour and seven days after contamination, and euthanized seven days later, total ^{238}Pu body content and distribution results at the different necropsy times were subjected to statistical analysis and are depicted in Figure 1. At treatment time points ranging from 1 hour to 16 hours post-contamination, a single parenteral dose of either 3,4,3-LI(1,2-HOPO) or 5-LIO(Me-3,2-HOPO) resulted in significant increases in ^{238}Pu elimination rates and total body burden and distinct tissue content reductions, as compared to

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4 saline-treated controls. However, only groups chelated with 3,4,3-LI(1,2-HOPO) at those time
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6 points lead to decreases in ^{238}Pu content that were significantly better than those observed after
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8 DTPA treatment. In addition, 3,4,3-LI(1,2-HOPO) was the only chelating option for which ^{238}Pu
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10 elimination was significantly enhanced when treatment was administered at delayed time points
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12 from 24 hours to 7 days after contamination. It was also the only ligand that resulted in
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14 significantly reduced ^{238}Pu skeleton content at all tested treatment time points. The decorporation
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16 efficacy decreased with increasing delays in treatment. Nevertheless, the elimination rates
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18 followed different kinetics depending on the time elapsed between contamination and treatment,
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20 as shown in Figure 2. While prompt chelation treatment (1 hour post-contamination) with
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22 HOPO ligands promoted the immediate excretion of more than 80% of the injected dose, the
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24 rates of elimination became slower than those observed in control animals by 2 or 3 days post-
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26 treatment for 5-LIO(Me-3,2-HOPO) or 3,4,3-LI(1,2-HOPO), respectively. The excretion
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28 enhancements promoted by delayed treatment (7 days post-contamination) were not as
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30 pronounced; however, the rates of elimination observed in the 7 days following treatment with
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32 either HOPO ligand were still comparable or faster than those observed in control animals.
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34 While 3,4,3-LI(1,2-HOPO) exhibited largely better efficacies than 5-LIO(Me-3,2-HOPO) at all
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36 time points, the rates of elimination were also notably different between both ligands: beyond the
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38 initial immediate action of the ligands observed within 24 hours after treatment, 5-LIO(Me-3,2-
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40 HOPO) did not display significant sustained action in contrast with 3,4,3-LI(1,2-HOPO) or
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42 DTPA. The cumulative excretion patterns shown in Figure 2 provide a clear visual representation
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44 of these differences. For treatment time points between 16 hours and 7 days, the sustained action
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46 of DTPA was visible over several days after treatment, with a curve-shaped excretion pattern,
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48 reaching similar (even higher in one case) excretion levels as with 5-LIO(Me-3,2-HOPO) by the
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fourth day post-treatment. Similarly, the prolonged efficacy of 3,4,3-LI(1,2-HOPO) was visible throughout the seven-day excreta collection period of this study, revealing 3,4,3-LI(1,2-HOPO) to be not only the most efficacious, but also the fastest treatment option to reduce ^{238}Pu contamination. Daily fecal and urinary excretion rates for each chelating treatment were also examined and are displayed in Figure 3. These panels show that independently of the post-contamination treatment time, both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) systematically enhanced excretion primarily through the biliary pathway, with only a minor urinary component, unlike DTPA treatment.

3.2 Prophylactic Parenteral or Oral Chelation Treatment

For this study where mice were treated prophylactically between 1 hour and 48 hours prior to contamination, total ^{238}Pu body content and distribution results at three days post-contamination were subjected to statistical analysis and are depicted in Figure 4. Parenteral administrations of 5-LIO(Me-3,2-HOPO) or DTPA were effective at significantly reducing body and tissue ^{238}Pu burden over a very short prophylactic window (1 hour and 6 hours, respectively), as shown in Figure 4, Panel A. In contrast, 3,4,3-LI(1,2-HOPO) exhibited remarkable prophylactic activity even when injected once 48 hours prior to contamination (Figure 4, Panel B). All animals showed large significant reductions in full body, skeleton, liver, soft tissue, and kidney content of ^{238}Pu for the groups treated parenterally with 3,4,3-LI(1,2-HOPO), compared to the corresponding saline- and DTPA-treated groups. As expected, decorporation efficacy decreased with increasing delays between treatment and contamination. Nevertheless, the efficacy level observed after a 3,4,3-LI(1,2-HOPO) injection 48 hours before contamination (up to 50% ^{238}Pu excreted within 3 days) also suggests that the prophylactic window could be extended further. The prophylactic efficacy of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) was also probed

after oral administration (Figure 4, Panel C). Groups treated with either ligand in the 6-hour prophylactic window showed significant reductions in total body, skeleton and liver content of ^{238}Pu compared to the saline control groups. However, the low oral bioavailability of 3,4,3-LI(1,2-HOPO) was reflected in the lack of decorporation efficacy for earlier treatments. Finally, daily fecal and urinary outputs after parenteral or oral 3,4,3-LI(1,2-HOPO) prophylactic administration are displayed in Figure 5. All panels show a rapid loss of elimination enhancement beyond the first day after contamination. For all treatment regimens but one, ^{238}Pu excretion is again predominantly fecal. However, in the case of parenteral treatment with 3,4,3-LI(1,2-HOPO) right before contamination, at the -1 hour time point, the ^{238}Pu level detected in the urine was 3-fold higher than the fecal output.

4. Discussion

The actinide decorporation efficacies of the two hydroxypyridinonate ligands 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) have been investigated through many *in vivo* studies over the past three decades [12, 13, 18, 19, 22], in order to delineate the therapeutic potential of both experimental compounds. These experiments were designed to characterize radionuclide elimination enhancement after prompt or slightly delayed treatment with chelating agents, most often within the first 24 hours of the contamination event. Scenarios of human contamination with actinides include a myriad of unknowns, but it unfortunately seems improbable that treatment would undergo wide distribution and be made available to a large population immediately post-incident. The first experiment described here was therefore planned to probe the extent of decorporation efficacy well beyond the first 24 hours of emergency response, with a delay of up to 7 days in treatment administration. In this case, extending the necropsy time points to 7 days post treatment was useful for the comparison of plutonium excretion patterns over

several days, i.e. until rates of actinide elimination slowed considerably. When preparing for a nuclear emergency, further considerations must be taken into account, including the need for pre-event prophylaxis, particularly for the military and first-responders. In the second study described in this report, a different experimental protocol was adopted to explore the potential prophylactic activity of the actinide decorporation agents under development. Treatment was administered either parenterally or orally at times preceding the ^{238}Pu challenge, with a delay of up to 48 hours prior to contamination. In this study, the necropsy time point was set at 3 days post-contamination, independent of the treatment time.

When provided after the contamination, and despite delayed treatment, parenteral 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO) and DTPA all clearly enhanced ^{238}Pu elimination through the investigated 7-day post-treatment period. However, the two HOPO ligands behaved distinctly in that the sustained excretion enhancement known and confirmed for 3,4,3-LI(1,2-HOPO) and DTPA over several days was not observed with 5-LIO(Me-3,2-HOPO). Furthermore, the rates of ^{238}Pu elimination observed after 5-LIO(Me-3,2-HOPO) or DTPA treatment slowed down earlier than those following 3,4,3-LI(1,2-HOPO) injections. The superiority of 3,4,3-LI(1,2-HOPO) in enhancing ^{238}Pu rates of elimination was also evidenced in the prophylactic protocol: while 5-LIO(Me-3,2-HOPO) and DTPA resulted in slight ^{238}Pu content reduction if administered within 6 hours prior to contamination, the efficacy of 3,4,3-LI(1,2-HOPO) was remarkably higher and observed even with treatments as early as 48 hours before the ^{238}Pu challenge. While the ^{238}Pu complexes formed with 3,4,3-LI(1,2-HOPO) or DTPA undergo relatively fast clearance and are fully expelled from the body within 24 hours [13], it has been suggested that the sustained excretion enhancement profiles in treated animals are due to intracellular uptake of the ligands and delayed clearance [23]. The pharmacokinetics and biodistribution profile of the ^{14}C -labeled

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4 3,4,3-LI(1,2-HOPO) were recently characterized in the young Swiss-Webster mouse model used
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6 here [24]. After parenteral injection, the radiolabeled compound was rapidly distributed into high
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8 vascular tissues, and highest kidney and liver concentrations were seen as early as 1-hour post-
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10 administration. However, those high concentrations stayed constant and more strikingly, about
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12 40% of the administered dose was still in various tissues and organs after 24 hours. These results
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14 were a good indicator of a longer residence time for 3,4,3-LI(1,2-HOPO) and correlate well with
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16 both its sustained action after treatment and large prophylactic window. Similarly, clearance was
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18 faster after oral administration of the radiolabeled ligand, as most of the compound remained
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20 unabsorbed and was simply excreted by 24 hours. Nevertheless, retention of the ligand was
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22 significant within the first 6 hours after oral administration, which also corroborates the shorter
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24 but consequent prophylactic activity of 3,4,3-LI(1,2-HOPO) after oral administration.
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31 The predominant biliary pathway observed in ^{238}Pu excretion promoted by either HOPO ligand
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33 is drastically different from the enhanced urinary excretion patterns resulting from treatment with
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35 DTPA, as evidenced by the illustration of the fecal and urinary ^{238}Pu outputs in Figure 3. Those
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37 differences have been discussed previously and are presumably based on respective ligand and
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39 actinide-complex physico-chemical parameters such as solubility, lipophilicity, and ionization
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41 constants [13, 18, 19]. In addition, the biliary pathway is the main mode of elimination for 3,4,3-
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43 LI(1,2-HOPO) in mice, as demonstrated by high accumulation of ^{14}C -labeled 3,4,3-LI(1,2-
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45 HOPO) in the feces after either parenteral or oral administration [24]. It is therefore likely that
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47 the radionuclide excretion path is ligand-driven. Of particular interest is the fact that a ^{238}Pu
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49 urinary output 3-fold higher than the fecal level was noted in the single case of 3,4,3-LI(1,2-
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51 HOPO) prophylactic parenteral treatment 1 hour prior to contamination. The disposition profiles
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53 of the ^{14}C -labeled ligand also displayed higher urinary excretion at early time points, within 4
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hours of ligand administration, due to the longer colonic transit times associated with biliary excretion. The excretion pattern observed in the early prophylactic case therefore suggests that the 3,4,3-LI(1,2-HOPO) ligand chelated the available ^{238}Pu fraction that had just entered the systemic circulation, preventing it from reaching the radionuclide deposition sites.

As detailed above, ^{238}Pu elimination enhancement was observed after parenteral 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO), or DTPA treatments delayed to up to 7 days post-contamination, with significant reductions in full body and tissue content in the case of 3,4,3-LI(1,2-HOPO). However, decorporation efficacy clearly decreases with increasing delays in treatment. Another attribute of the 3,4,3-LI(1,2-HOPO) ligand uncovered in these studies is its high decorporation efficacy when administered prophylactically as early as 48 hours pre-exposure. While, it is remarkable that the lead hydroxypyridinonate chelating agent under development, 3,4,3-LI(1,2-HOPO) promotes significant removal of ^{238}Pu from contaminated mice even when a single treatment is delayed to 7 days after exposure, the results of this study confirm that decorporation treatments should be administered with the shortest possible delay post-contamination. In addition, the long prophylactic window of activity revealed here opens a new perspective in the indication and use of this decorporation treatment. Extensive non-clinical studies have highlighted the safety of hydroxypyridinonate chelating agents [17, 19, 25] and, in the absence of serious toxicity concerns, it is now a possibility that 3,4,3-LI(1,2-HOPO) be considered useful for prophylactic use, in which treatment decisions will be made based on suspicion or prevention of potential contamination rather than on knowledge that may require long triage, estimation, and decision-making procedures in emergency settings. This study therefore complements the available efficacy data sets for hydroxypyridinonate ligands in

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4 general and 3,4,3-LI(1,2-HOPO) in particular, and combined with parallel clinical safety
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6 analyses, will help define therapeutic options for ^{238}Pu decorporation.
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Figures

Figure 1. Total ^{238}Pu body content and distribution at 7 days after a single time-delayed ip chelation treatment. Young adult female Swiss-Webster mice injected iv with ^{238}Pu -citrate; saline or treatment (3,4,3-LI(1,2-HOPO) [30 $\mu\text{mol/kg}$], 5-LIO(Me-3,2-HOPO) [100 $\mu\text{mol/kg}$], or Ca-DTPA [30 $\mu\text{mol/kg}$] administered ip at 1 h, 5 h (A), 16 h, 24 h (B), 3 d, or 7 d (C) post-contamination; mice euthanized 7 days after treatment. Data expressed as percent of injected ^{238}Pu dose (% ID, mean $\pm$ SD) for each five-mouse group. Groups with significantly lower retention than for control mice are indicated by * or ** ($p < 0.05$ or $p < 0.01$, 1-way ANOVA with post hoc Dunnett's multiple comparison test), while groups with significantly lower retention than for Ca-DTPA-treated mice are indicated by # or ## ($p < 0.05$ or $p < 0.01$, 1-way ANOVA with post hoc Tukey's HSD multiple comparison test).

Figure 2. Daily ^{238}Pu cumulative excretion after a single time-delayed ip chelation treatment at 1 h (A), 5 h (B), 16 h (C), 24 h (D), 3 d (E), or 7 d (F) post-contamination. Young adult female Swiss-Webster mice injected iv with ^{238}Pu -citrate; saline or treatment (3,4,3-LI(1,2-HOPO) [30 $\mu\text{mol/kg}$], 5-LIO(Me-3,2-HOPO) [100 $\mu\text{mol/kg}$], or Ca-DTPA [30 $\mu\text{mol/kg}$]) administered ip at 1 h, 5 h, 16 h, 24 h, 3 d, or 7 d post-contamination as indicated by the arrows; mice euthanized 7 days after treatment. Excreta of each five-mouse group were pooled and standard deviations are not available as the data are calculated from the cumulative collected excretion.

Figure 3. Daily ^{238}Pu fecal (top panels **A**, **B**, **C**) and urinary (bottom panels **D**, **E**, **F**) output after a single 3,4,3-LI(1,2-HOPO) (**A** and **D**), 5-LIO(Me-3,2-HOPO) (**B** and **E**), or DTPA (**C** and **F**), time-delayed ip chelation treatment at 1 h, 5 h, 16 h, 24 h, 3 d, or 7 d post-contamination. Young adult female Swiss-Webster mice injected iv with ^{238}Pu -citrate; saline or treatment (3,4,3-LI(1,2-HOPO) [30 $\mu\text{mol/kg}$], 5-LIO(Me-3,2-HOPO) [100 $\mu\text{mol/kg}$], or Ca-DTPA [30 $\mu\text{mol/kg}$]) administered ip at 1 h, 5 h, 16 h, 24 h, 3 d, or 7 d post-contamination; mice euthanized 7 days after treatment. Excreta of each five-mouse group were pooled daily and standard deviations are not available as the data are calculated from the group-collected excretion.

Figure 4. Total ^{238}Pu body content and distribution 3 days after a contamination event preceded by a single prophylactic chelation treatment. Young adult female Swiss-Webster mice injected iv with ^{238}Pu -citrate; saline or treatment (3,4,3-LI(1,2-HOPO) [30 $\mu\text{mol/kg}$ ip or 100 $\mu\text{mol/kg}$ po], 5-LIO(Me-3,2-HOPO) [100 $\mu\text{mol/kg}$ ip or 200 $\mu\text{mol/kg}$ po], or Ca-DTPA [30 $\mu\text{mol/kg}$ ip]) administered ip (**A** for 5-LIO(Me-3,2-HOPO) and DTPA, and **B** for 3,4,3-LI(1,2-HOPO)) or po (**C**) at 1 h, 6 h, 16 h, 24 h, 30 h, 40 h, or 48 h prior to contamination; mice euthanized 3 days after contamination. Data expressed as percent of injected ^{238}Pu dose (% ID, mean $\pm$ SD) for each five-mouse group. Groups with significantly lower retention than for control mice are indicated by * or ** ($p < 0.05$ or $p < 0.01$, 1-way ANOVA with post hoc Dunnett's multiple comparison test), while groups with significantly lower retention than for Ca-DTPA-treated mice are indicated by # or ## ($p < 0.05$ or $p < 0.01$, 1-way ANOVA with post hoc Tukey's HSD

multiple comparison test). In Panel **B**, all distinct tissue content bars should indicate $^{**\cdot\#\#}$ (-1 h to -24 h) or ** (-30 h to -48 h), but symbols were omitted for clarity.

Figure 5. Daily ^{238}Pu fecal (top panels **A** and **B**) and urinary (bottom panels **C** and **D**) output after a single 3,4,3-LI(1,2-HOPO) prophylactic ip (left panels **A** and **C**) or po (right panels **B** and **D**) chelation treatment. Young adult female Swiss-Webster mice injected iv with ^{238}Pu -citrate; 3,4,3-LI(1,2-HOPO) [30 $\mu\text{mol/kg}$ ip or 100 $\mu\text{mol/kg}$ po] administered ip or po at 1 h, 6 h, 16 h, 24 h, 30 h, 40 h, or 48 h prior to contamination; mice euthanized 3 days after contamination. Excreta of each five-mouse group were pooled daily and standard deviations are not available as the data are calculated from the group-collected excretion.