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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 parenteral or oral treatment administration, i.e., well beyond the initial 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 h 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

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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; as a result of its low absorption in the gastrointestinal tract, it needs to be administered either intravenously or via nebulized inhalation depending on the route of contamination; and it must be taken in large quantities [7–9]. Experiments have also shown that Ca-DTPA does not chelate plutonium significantly after the element's deposition in organs, explaining the necessity of administering treatment as soon as possible post-contamination [10]. However, although a large molar percentage of DTPA administered parenterally can be accounted for in blood and extracellular fluid, a small fraction can reach intracellular spaces, responsible for the liver decorporation efficacy, as demonstrated in rats and dogs [11,12]. 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 others, such as isotopes of uranium, neptunium, and europium [13–15]. 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 [16–18] since efficacy trials in human beings cannot be ethically conducted [19–21]. 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 treatments 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, contamination with soluble ^{238}Pu-citrate was performed through a single intravenous injection, and the chosen animal model was the young adult female Swiss-Webster mouse. An advantage of these procedures is the need for only small amounts of radiological contaminant to obtain accurate counting statistics in tissue and excreta samples, avoiding large radionuclide inventories and reducing the amount of handled radioactive materials. However, one important limitation of this single mouse model is the difference in biliary outlets for actinides observed in different species, which warrants additional pivotal efficacy studies performed in a second animal species, as mandated by the FDA's Animal Rule.

2. Materials and methods

2.1. Contaminant and ligand 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 0.74 kBq (1.16 ng) of ^{238}Pu in 0.008 M sodium citrate and 0.14 M NaCl, pH 4. The ligands 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 [19]. DTPA was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was formulated as Ca-DTPA using $CaCO_3$ and NaOH, similar to the formerly available drug product commercialized by Hameln Pharmaceuticals gmbh (Hameln, Germany). Ligand solutions were prepared such that the selected dosages (30 μmol/kg for Ca-DTPA, 30 or 100 μmol/kg for 3,4,3-LI(1,2-HOPO), and 100 or 200 μmol/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 [22].

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 25–40% of their total blood volume) have been determined previously [23]. Mice were kept under a 12-h 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. Treatment dose volumes were adjusted based on the weight of the mouse, with a 0.5 mL volume corresponding to a 35 g mouse. 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

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