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Dose-Dependent Efficacy and Safety Toxicology of Hydroxypyridinonate Actinide Decorporation Agents in Rodents: Towards a Safe and Effective Human Dosing Regimen

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Abstract

Two hydroxypyridinone-containing actinide decorporation agents, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), are being developed for the treatment of internal actinide contamination by chelation therapy. Dose-response efficacy profiles in mice were established for the removal of intravenously injected ²³⁸Pu and ²⁴¹Am after parenteral and oral treatment with these chelators. In both cases, presumed efficacious doses promoted substantially greater actinide elimination rates than the currently approved agent, diethylenetriamine-pentaacetic acid, considering two different interspecies scaling methods for the conversion of human doses to equivalent rodent dose levels. In addition, genotoxicity of both ligands was assessed using the *Salmonella/Escherichia coli* microsome plate incorporation test and the Chinese hamster ovary cell chromosome aberration assay, showing that neither ligand is genotoxic, in the presence and absence of metabolic activation. Finally, maximum tolerated dose studies were performed in rats for seven consecutive daily oral administrations with the chelators, confirming the safety of the presumed efficacious doses for 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO). The results of these studies add to the growing body of evidence that both decorporation agents have remarkable decorporation efficacy properties and promising safety toxicology profiles. These results are necessary components of the regulatory approval process and will help determine the optimal human dosing regimens for the treatment of internal radionuclide contamination.

INTRODUCTION

Exposure to radionuclides in the context of a nuclear accident or terrorist attack presents a risk with potentially devastating health consequences. The actinides plutonium (Pu), americium (Am), curium (Cm), uranium (U) and neptunium (Np) are all radioactive, mostly decay by α -particle emission, and may be released into the immediate environment following a disaster (1). Once internalized, the radionuclides would be distributed to various tissues with patterns that depend on the chemical and physical form of the contaminant in question (2). The densely ionizing α particles emitted by actinides tend to get retained in bone and liver, and in the lungs if inhaled. This leads to dose-dependent tissue damage and cancer, and can cause manifestations of acute radiation syndrome (3). The only practical

therapy to reduce the health consequences of internal actinide contamination is aggressive, and often protracted, treatment with small molecules that can form excretable chelates (4, 5).

Chelation therapy with intravenous calcium trisodium (Ca)- and zinc trisodium (Zn)-diethylenetriamine pentaacetic acid (DTPA) was approved by the U.S. Food and Drug Administration (FDA) in 2004 for the treatment of individuals with known or suspected contamination with Pu, Am or Cm (6) based on historical decorporation results from several animal species and documented chelation treatments in isolated human contamination accidents (7–10). However, Ca-DTPA and Zn-DTPA in the current approved formulation can only be administered intravenously or through a nebulizer and are contraindicated for U chelation because of potential added risks of nephrotoxicity (11).

Two hydroxypyridinone-based decorporation ligands, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO), have emerged as promising therapeutic candidates as a result of multiple decorporation efficacy studies in several animal species (4, 5, 10, 12). When compared with Ca-DTPA, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) have superior efficacy in chelating actinides and have been shown to sequester a wider spectrum of radionuclides including U and Np (12). In addition, these hydroxypyridinone decorporation ligands have been shown to be highly efficacious when administered orally (13), a highly desirable route of administration from a logistical standpoint in a mass casualty setting. However, before approval can be granted for human therapeutic use of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) as radionuclide decorporation agents, *in vivo* efficacy and safety, as well as feasibility of large-scale production and storage, must be rigorously demonstrated, following guidelines from the regulatory agencies (14–16). Evaluation of these ligands falls under the Animal Efficacy Rule (15), which allows for approval of a new drug based on (1) efficacy data from more than one animal species, and (2) safety data from animals and normal human volunteers. Efficacy would thus only be demonstrated in animals because controlled clinical trials, in which actinides were administered to humans for the sole purpose of decorporation tests, would be unethical. There is currently no precedent for the approval of a new radionuclide decorporation agent under the Animal Efficacy Rule although the FDA issued a guidance document that details their current thinking on the topic (14).

The superior decorporation efficacy of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) in several animal species (4, 5, 10, 12), the pharmacokinetic profile of these ligands in Sprague-Dawley rats (17), and the results from preclinical safety studies conducted under good laboratory practice (GLP) where Sprague-Dawley rats were orally administered these ligands for 28 days (12) have all been previously described. Herein we discuss additional rodent efficacy and preclinical safety results that have arisen as these decorporation agents progress down the drug development pathway. Specifically, we report (1) that 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) chelate Am and Pu in a dose-responsive manner after parenteral and oral administration in female Swiss-Webster mice with superior efficacy when compared to control mice that were administered Ca-DTPA, (2) that both ligands are not genotoxic in the FDA-required GLP genetic toxicology bacterial reverse mutation Ames assay and the *in vitro* chromosomal aberration assay in Chinese hamster ovary (CHO) cells, and (3) the maximum tolerated dose (MTD) safety results in male Sprague-Dawley rats after 7 consecutive days of oral administration. The results of these studies add to the growing body of evidence that 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) both have remarkable decorporation efficacy properties and promising safety toxicology profiles.

MATERIALS AND METHODS

General

All chemicals were obtained from commercial suppliers and used as received. The test articles 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were prepared by Albany Molecular Research, Inc. (Albany, NY), Synthetech, Inc. (Albany, OR), Starks Associates (Buffalo, NY) or Ash Steven's Inc. (Detroit, MI), as described previously (12). The general procedures for animal care and housing were conducted in accordance with the National Research Council for the Care and Use of Laboratory Animals and the Animal Welfare Standards (18). All procedures and protocols used in the described *in vivo* studies were reviewed and approved by the Institutional Animal Care and Use Committees of Lawrence Berkeley National Laboratory or SRI International, and were performed in AAALAC accredited facilities.

Efficacy Studies in Mice

Test article solutions were prepared such that the selected dose [ranging from 0.03 to 500 $\mu\text{mol/kg}$ for $\text{CaNa}_3\text{-DTPA}$, 0.01 to 200 $\mu\text{mol/kg}$ for 3,4,3-LI(1,2-HOPO) and 0.01 to 500 $\mu\text{mol/kg}$ for 5-LIO(Me-3,2-HOPO)] was contained in 0.5 ml of 0.14 *M* NaCl. The pH of the dosing solutions were adjusted to 7.4–8.4 with sodium hydroxide. All solutions were filter-sterilized (0.22 μm) prior to administration. Multiple groups of five female Swiss-Webster mice (13–14 weeks old, 26.1–33.0 g; Simonsen Laboratories, Gilroy, CA) were used for each experiment. 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. All mice were given water and food *ab libitum* until the start of the study. Some groups of mice were fasted for 16 h prior to treatment, while others were maintained under normal diet for the duration of the study. Under isoflurane anesthesia, 0.2 ml of an actinide solution was injected intravenously (i.v.) into the lateral tail vein of each animal. Radioactivities and metal masses of the actinide solutions in 8 *mM* sodium citrate were ^{238}Pu (0.74 kBq, 1.2 ng) or ^{241}Am (0.43 kBq, 3.7 ng in injection experiments; 0.65 kBq, 5.5 ng in oral experiments); ligands were administered by intraperitoneal (i.p.) injection to normally fed mice or orally (gastric intubation, po) to fasted mice 1 h after the actinide administration. Food was provided to the fasted mice 4 h after the actinide injection. All animals were euthanized 24 h after the actinide injection. Details of sample collection, preparation, radioactivity measurements and data reduction have been published previously (19, 20). All individual samples were mixed with Ultimagold (Perkin Elmer Corporation, Shelton, CT) for detection of the radiotracers, ^{238}Pu and ^{241}Am , by liquid scintillation counting (Packard Tri-Carb model B4430; Perkin Elmer). Metabolic balance calculations of ^{238}Pu or ^{241}Am were conducted for each study. In most cases, the ^{238}Pu and ^{241}Am recovered was higher than 95% and 97% of the amount injected, respectively, and data were normalized to 100% recovery. The corrected, experimental data are expressed as percentage of injected dose (%ID) and values are arithmetic means $\pm$ SD. When comparing values between groups, 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 (99% confidence interval level) was used to compare groups of animals treated with a chelating agent to the corresponding control group that was administered saline. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). Dose-response curves were fitted by sigmoidal logistic analysis using Origin 6.1 (OriginLab, Northampton, MA).

Genotoxicity Studies

The *in vitro* studies *Salmonella-Escherichia coli*/microsome plate incorporation test (Ames test), and CHO cell chromosomal aberration assay were conducted in compliance with GLP and the International Conference on Harmonization (ICH) Guidelines (21–23).

Experiments with *Salmonella typhimurium* LT2 strains (TA1535, TA1537, TA98 and TA100, provided by Dr. Bruce Ames, University of California at Berkeley) and *E. coli* strain WP2 *uvrA* (obtained from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland) were performed as described previously (24–26), using the standard plate incorporation procedure. Positive controls, with and without rat liver homogenate metabolic activation (S9) were included in each assay. For each ligand, a dose range finding and two mutagenicity experiments were conducted in the presence or absence of S9. The maximum concentration used for each ligand was 5 mg/plate and triplicate plates were used for each concentration. Each ligand was prepared on the day of use in sterile water as a 50 mg/ml stock solution. The pH of the solution was adjusted with sodium hydroxide to a final pH of 6.8–7.0. After sonication, serial dilutions were performed to yield the tested final concentration of 39.1–5,000 µg/plate. Dose levels and homogeneity were confirmed by high-performance liquid chromatography, using a previously described method (12). Statistical analyses were performed using the SAS analysis system (v. 9.1 and 6.12; SAS Institute, Inc., Cary, NC). A positive Ames test is defined as a reproducible and statistically significant (one-tailed Dunnett's *t* test, *P* < 0.01) increase in the number of revertants at one or more tested concentrations and if dose-related increases in the number of revertants were observed by regression analysis (*t* statistic).

Chromosome aberration experiments were conducted in CHO cell cultures (ATCC CCL 61 CHO-K1, proline-requiring, obtained from the American Type Culture Collection, Rockville, MD). For each ligand, an initial cytotoxicity experiment followed by two chromosome aberration experiments was conducted in the presence and absence of rat S9. Standard procedures were used for cell propagation, harvesting cultures for metaphase spreads, and analyzing aberrations (24, 27, 28). Individual cultures of cells were exposed to either ligands, negative or positive controls for 3 and 21 h without S9 and 3 h with S9. Two positive controls, methyl methanesulfonate (MMS) and cyclophosphamide were used for each experiment. Cultures were exposed to 50 µg/ml of MMS for 3 h with S9 and 20 or 30 µg/ml for 21 h without S9. Cultures were also exposed to 12.5 µg/ml of cyclophosphamide with S9.

All the ligand solutions were prepared within a day of use and included pH adjustment to pH 6.8–7.0 with sodium hydroxide. Stock solutions of 3,4,3-LI(1,2-HOPO) were prepared in sterile water at 50 mg/ml. Stock solutions were serially diluted in sterile water to make the final concentrations of 31.25, 62.5, 125, 250 and 500 µg/ml (corresponding to 40, 80, 160, 330 and 650 µM, respectively) in culture media for 3 h exposure with or without S9 and at 62.5, 125 and 250 µg/ml for 21 h without S9. For 5LIO(Me-3,2-HOPO), a stock solution of 8 mg/ml was prepared and serially diluted in sterile water for analysis at 50, 100, 200 and 400 µg/ml culture media (corresponding to 125, 250, 500 and 1000 µM, respectively). Dose levels and homogeneity were confirmed by high-performance liquid chromatography (HPLC) (17). Duplicate cultures were maintained for each concentration and for each ligand. For each concentration, 1,000 cells for toxicity and 200 cells for metaphases were scored and recorded using software program CA Score v.1.3, CA Talley V.1.2, CA Sum v. 1.3 developed by SRI International in a blinded manner for the numbers of cells with chromosome aberrations and included cells with chromatid and chromosome type aberrations. For statistical analysis, the number of cells with structural chromosome damage observed in the ligand and the positive control treatment groups were compared with those of the concurrent vehicle control group. Chromatid and chromosome gaps were not included

in the analysis. A test article would be considered positive if there was a statistically significant increase (Fisher's exact test, $P < 0.05$ one-tailed, FISHEX.PL.XLT v. 1.0 macro on MS Excel) in the frequency of cells with structural chromosomal damage at one or more concentrations, and this increase was dose-related and reproducible using the Cochran-Armitage test (COCHRAN 1.22) for trends in binomial proportions at $P < 0.01$.

Maximum Tolerated Dose Studies in Rats

A total of 27 male Sprague-Dawley rats (Harlan, Livermore, CA), at 6–8 weeks of age, housed 3 per microisolator cage, were maintained on Purina Certified Rodent Chow 5002 (Richmond, IN) and reverse osmosis purified tap water *ad libitum*, under controlled lighting (12 h light-dark cycle). After a three-day quarantine period, body weights were measured at randomization, and animals were assigned to dose groups (3 rats per group). Experiments with orally administered 3,4,3-LI(1,2-HOPO) or 5-LIO(Me-3,2-HOPO) were conducted separately but with identical study designs. The dose levels of 3,4,3-LI(1,2-HOPO) used were 532, 932, 1,332 or 1,732 $\mu\text{mol/kg/day}$ (400, 700, 1,000 or 1,300 mg/kg/day , respectively). Formulations of 3,4,3-LI(1,2-HOPO) were clear and colorless and required a pH adjustment with sodium hydroxide to obtain a final pH of 5.18–5.55. The dose levels of 5-LIO(Me-3,2-HOPO) used were 500, 1,000 or 2,000 $\mu\text{mol/kg/day}$ (203, 406 or 816 mg/kg/day , respectively). Formulated material of 5-LIO(Me-3,2-HOPO) was a white suspension at pH 5.8. The solution was stirred and sonicated to ensure homogeneity. The vehicle used was Dulbecco's phosphate buffered saline (PBS, Sigma Aldrich, St. Louis, MO). All solutions were protected from light, stored refrigerated and brought to room temperature before being administered to the animals. All formulated dosing solutions were used within 4 to 7 days of preparation.

Animals were treated once a day for 7 consecutive days. Dose level selection was based on previous 28-day repeat dose safety studies (12) and a single dose range-finding experiment. Dose volumes were delivered at 15 ml/kg with a plastic, round-tipped, gavage needle. Body weights were measured on days 1 and 8. Detailed clinical observations were conducted once a day at 2–4 h after dose administration. On day 8, blood was collected to evaluate hematology and serum chemistry parameters using standard methods (Advia 120 Analyzer, Bayer HealthCare, Tarrytown, NY and Cobas c-501 Analyzer, Roche Diagnostics, Indianapolis, IN) just before i.p. administration of pentobarbital. All organs and external body orifices were examined during necropsy. Statistically significant changes in body weight and clinical pathology parameters were evaluated using one-way ANOVA followed by Dunnett's test ($P < 0.05$ and $P < 0.01$; Labcat in-life v.8.2, clinical pathology v.4.42, Lawrenceville, NJ). Fourteen tissues from each animal including brain, colon, esophagus, duodenum, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph nodes, spleen, stomach, and urinary bladder were formalin-fixed. All collected tissues from control and high dose treated rats [1,332 and 1,732 $\mu\text{mol/kg/day}$ for 3,4,3-LI(1,2-HOPO) and 2,000 $\mu\text{mol/kg/day}$ for 5-LIO(Me-3,2-HOPO)] and from any animals with unscheduled deaths were embedded in paraffin, cut approximately 5- μm thick, and stained with hematoxylin and eosin for histopathologic examination by a board-certified veterinary pathologist. In the event that target organs of toxicity were identified in the high dose groups, the tissues from the same organs in the lower dose groups were subsequently processed and also evaluated by the histopathologist to determine the maximally tolerated dose for each ligand.

RESULTS

Dose-Response Efficacy Studies in Mice

A series of Pu and Am decorporation efficacy studies was performed in mice with the two hydroxypyridinonate ligands, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO). The

treatment protocols were designed to span a range of parenteral and oral treatment doses and to establish dose-response profiles for both ligands in comparison with Ca-DTPA. The in-life portions of these studies were accomplished without incident. The excretion and distribution of retained Pu and Am 24 h after i.v. injection of soluble ^{238}Pu citrate or ^{241}Am -citrate (and 23 h after administration of the decorporation agent) were expressed as percentage of injected dose (%ID) and are shown in Tables 1, 2 and 3, while total body retention is illustrated in Fig. 1. The dose-response profiles for parenteral treatment with the three chelators after ^{238}Pu -citrate contamination have been described and discussed before (20, 29), and the corresponding total body retention data is reproduced in Fig. 1A for comparison purposes. The data presented here were fitted by nonlinear sigmoidal logistic analysis, providing portions of typical pharmacological dose-response profiles that can be interpolated or extrapolated for efficacy assessment at doses outside of the tested ranges (Fig. 1).

As illustrated in Fig. 1B, 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO) and Ca-DTPA, when given orally at doses >3 , 30 and 100 $\mu\text{mol/kg}$, respectively, significantly reduced the total ^{238}Pu body burden when compared to saline-administered controls. The hydroxypyridinonate chelators reached a decorporation plateau corresponding to the removal of approximately 80% of the injected ^{238}Pu at oral dose levels 100 $\mu\text{mol/kg}$ for 3,4,3-LI(1,2-HOPO) and 500 $\mu\text{mol/kg}$ for 5-LIO(Me-3,2-HOPO). In contrast, a decorporation plateau was not reached at the tested oral doses of Ca-DTPA, with a maximum removal of approximately 40% of ^{238}Pu at the highest Ca-DTPA oral dose of 500 $\mu\text{mol/kg}$. In addition, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) significantly reduced the discrete ^{238}Pu content in all analyzed tissues (skeleton, liver, kidneys and remaining soft tissues) at oral doses >10 and 100 $\mu\text{mol/kg}$, respectively (Table 1). The maximum efficacy of ^{238}Pu removal after oral administration of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) was similar to that previously reported after i.p. administration (Fig. 1A), with approximately 80% elimination for single parenteral doses 1 $\mu\text{mol/kg}$ for 3,4,3-LI(1,2-HOPO) and 10 $\mu\text{mol/kg}$ for 5-LIO(Me-3,2-HOPO) (20, 29). As expected, the efficacious dose of each ligand given through the i.p. route of administration was much lower than when the ligands were orally administered.

The decorporation agents were also highly effective at chelating Am. Specifically, all tested i.p. doses of 3,4,3-LI(1,2-HOPO) (from 0.3 to 200 $\mu\text{mol/kg}$) and 5-LIO(Me-3,2-HOPO) (from 1 to 500 $\mu\text{mol/kg}$), significantly reduced the ^{241}Am total body and liver contents when compared to 3 $\mu\text{mol/kg}$ of Ca-DTPA and the saline-administered controls. The ^{241}Am skeleton content was also significantly reduced after parenteral treatment with all doses of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) >10 $\mu\text{mol/kg}$ and Ca-DTPA doses >30 $\mu\text{mol/kg}$. Overall, the hydroxypyridinonate chelators were more potent than Ca-DTPA, but all three agents were capable of eliminating $>70\%$ of the injected ^{241}Am (Fig. 1C). The maximum decorporation efficacy was obtained for a single parenteral dose 60 $\mu\text{mol/kg}$ for 3,4,3-LI(1,2-HOPO) and 200 $\mu\text{mol/kg}$ for 5-LIO(Me-3,2-HOPO).

Oral doses >1 , 10 and 10 $\mu\text{mol/kg}$ of 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO) and Ca-DTPA, respectively, also significantly reduced the total ^{241}Am body content when compared to saline-administered controls. The maximum decorporation efficacy was reached at a single oral dose of 200 $\mu\text{mol/kg}$ for 5-LIO(Me-3,2-HOPO). However, maximum decorporation efficacy was not reached with the oral doses of 3,4,3-LI(1,2-HOPO) that were tested since no plateau was observed in the dose-response profile up to 200 $\mu\text{mol/kg}$ (Fig. 1D). We therefore predict that even better efficacy for the removal of ^{241}Am may be achieved with oral doses of >200 $\mu\text{mol/kg}$ of 3,4,3-LI(1,2-HOPO). Although a decorporation plateau was not reached at the tested oral doses of Ca-DTPA, our results show that only approximately 30% of ^{241}Am was removed at the highest Ca-DTPA

oral dose of 500 $\mu\text{mol/kg}$. Finally, both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) significantly reduced the liver ^{241}Am content at oral doses $>10 \mu\text{mol/kg}$, and treatment with 3,4,3-LI(1,2-HOPO) significantly reduced the skeleton ^{241}Am content at oral doses $>60 \mu\text{mol/kg}$.

These results show that parenteral and oral treatments with 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO), and Ca-DTPA prompted dose-dependent elimination rates and reductions of total body radionuclide burden with distinct tissue specificities for both Pu and Am. In particular, the hydroxypyridinonate ligands displayed substantially higher potency than Ca-DTPA used in the currently approved formulation format, and that oral treatment with 3,4,3-LI(1,2-HOPO) or 5-LIO(Me-3,2-HOPO) resulted in higher actinide-specific elimination than parenteral Ca-DTPA treatment.

Genotoxicity Assessments

Both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were evaluated for their mutagenic potential in the bacterial reverse mutation Ames test and for their ability to cause chromosome aberrations such as chromatid and chromosome gaps, breaks, exchanges, deletions, acentric fragments and dicentrics in CHO cells. These standardized, *in vitro* genotoxicity tests are part of a battery of tests required by the FDA to support eventual multiple dose clinical safety trials with both radionuclide decorporation agents (22).

The Ames test was performed for 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) in the dose range of 39.1 to 5,000 $\mu\text{g/plate}$ using five tester bacterial strains with and without the presence of rat liver homogenates (S9) to provide metabolic activation. The results are presented in Table 4 for 3,4,3-LI(1,2-HOPO) and Table 5 for 5-LIO(Me-3,2-HOPO). Cytotoxicity, evident by decreased revertant colony counts or the appearance of pinpoint non-revertant colonies, was only seen with the *E. coli* WP2 (*uvrA*) strain at doses 625 $\mu\text{g/plate}$ with or without S9 for 3,4,3-LI(1,2-HOPO) and at doses 1250 $\mu\text{g/plate}$ and 2,500 $\mu\text{g/plate}$ with and without S9, respectively, for 5-LIO(Me-3,2-HOPO). The positive control results were acceptable for all experiments, as they elicited a response fivefold increase over the mean value for the vehicle. Results for the negative controls were within $\pm 10\%$ of historical values for spontaneous revertants (data not shown). Slight but statistically significant ($P < 0.01$) increases in the number of revertant colonies in strain TA98 were observed for 3,4,3-LI(1,2-HOPO) and in strain TA100 were observed for 5-LIO(Me-3,2-HOPO) in the absence of S9 in one experiment. Log-log regression analysis suggests the increases to be dose-dependent. No other test conditions elicited revertant colony counts that were statistically significant. Since the increases in the number of revertant colonies for strains TA98 for the 3,4,3-LI(1,2-HOPO) and TA100 for 5-LIO(Me-3,2-HOPO) were not reproducible, increased $< \text{twofold}$ above the vehicle control, and well within the historical spontaneous revertant colony range when positive by regression analysis, these results were not considered to be a mutagenic response or biologically relevant. Thus, both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) were non-mutagenic in the bacterial reverse mutation Ames assay.

In the *in vitro* chromosomal aberration assay in CHO cells, cytotoxicity (corresponding to 50% reduction in mitotic index) was observed with 3,4,3-LI(1,2-HOPO) at 1,000 and 5,000 $\mu\text{g/ml}$ with and without S9 for 3 h and without S9 for 21 h. Cytotoxicity was observed with 5-LIO(Me-3,2-HOPO) at 800 $\mu\text{g/ml}$ for all exposures and S9 conditions. Results from the third to fourth highest scorable dose levels for each ligand were analyzed and are presented in Figs. 2 and 3. Since there was no significant increase in chromosome aberrations in the presence of S9 for 3 h, the results from two experiments were pooled and presented. Positive controls produced 26.4 to 38% aberrations. These values were statistically significant ($P < 0.05$) when compared to those obtained from the vehicle controls. For 3,4,3-LI(1,2-HOPO),

there was no significant increase in chromosome aberrations at 125, 250 and 500 $\mu\text{g/ml}$ for 3 h exposure and at 62.5, 125 and 250 $\mu\text{g/ml}$ for 21 h in the absence of S9 when compared with respective controls. In the presence of S9, there was no significant increase in CA at 125, 250 and 500 $\mu\text{g/ml}$ compared with controls. For 5-LIO(Me-3,2-HOPO), there was also no significant increase in chromosome aberrations at 100, 200 and 400 $\mu\text{g/ml}$ in the absence of S9 for 3 and 21 h and in the presence of S9 for 3 h exposure when compared with respective controls. The polyploidy index in treated cultures was 0.0 to 2.9% compared with 0.0 to 1.9% in controls. Moreover, there was no dose-related increase in polyploidy derived from cultures treated with either ligand. Thus, neither 3,4,3-LI(1,2-HOPO) nor 5-LIO(Me-3,2-HOPO) were considered clastogens at the noncytotoxic dose levels in CHO cells.

Maximum Tolerated Dose Studies in Rats

Determining the maximum tolerated dose (MTD) in rodents is an important and early *in vivo* safety test for potential therapeutics. Short-term MTD studies help determine the dose-limits for longer-term studies and guide the setting of the starting dose levels for non-rodent safety studies. Therefore, 7-day repeat dose studies were conducted in Sprague-Dawley male rats using relatively high-doses of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO).

Dose dependent toxicity was seen following oral gavage administration of 3,4,3-LI(1,2-HOPO) for 7 consecutive days to male rats at 532, 932, 1,332 and 1,732 $\mu\text{mol/kg/day}$ (400, 700, 1,000 and 1,300 mg/kg/day). Three animals were included in each dose group. Hunched posture, ruffled fur and tachypnea (rapid breathing) were observed beginning on day 4 in the highest 1,732 $\mu\text{mol/kg}$ dose group. Hunched posture and tachypnea were also apparent on days 7 and 8 in one of three rats in the lower 1,332 $\mu\text{mol/kg}$ dose group while all of the animals in the lowest two dose groups (532 and 932 $\mu\text{mol/kg}$) appeared normal throughout the 8 day study. No test article-related deaths were observed in these studies.

A statistically significant difference in body weight gain was observed in the highest dose group treated with 3,4,3-LI(1,2-HOPO). Rats in this group gained 25.5 ± 6.36 g over the course of the 8 day study while the controls gained 43.0 ± 2.00 g. No significant difference in body weight and weight gain were observed in the other dose groups. Evaluation of standard hematology and serum chemistry parameters revealed statistically significant dose dependent decreases in absolute and percent reticulocyte levels in the two highest dose groups when compared with controls (Fig. 4). Slight (<1.2 fold) but statistically significant increases in serum phosphorus were observed in all but the 932 $\mu\text{mol/kg}$ dose group and circulating calcium levels were slightly increased (<1.1 fold) in the 1,332 $\mu\text{mol/kg}$ group. All other hematology and clinical chemistry parameters appeared normal.

Histopathology changes were observed in the stomachs of all 3,4,3-LI(1,2-HOPO)-treated rats while none of the other 13 major tissues examined per animal had findings attributed to 3,4,3-LI(1,2-HOPO). Specifically, multifocal to diffuse, mild to moderate epithelial hyperplasia and focal to diffuse, minimal to moderate, hyperkeratosis were present in the nonglandular stomach of all rats in the treated groups and absent from all vehicle control rats. In addition, focal or multifocal, minimal to moderate submucosal edema of the glandular stomach was present in at least one rat from each of the 3,4,3-LI(1,2-HOPO) treated groups and absent in the vehicle control rats. Hyperkeratosis of the nonglandular stomach was dose-related (i.e., most prominent in the high dose groups) while the epithelial hyperplasia in the nonglandular stomach and sub-mucosal edema in the glandular stomach were not dose-related. Sub-mucosal minimal to moderate inflammation of the glandular and nonglandular stomach was observed in all rats, including those in the vehicle control group, and thus cannot be unequivocally attributed to 3,4,3-LI(1,2-HOPO). The MTD for 7-day oral administration of 3,4,3-LI(1,2-HOPO) to male rats is therefore estimated to be <532

$\mu\text{mol/kg/day}$, the lowest dose level tested in this study, and was assigned based on the identification of stomach abnormalities consisting of epithelial hyperplasia, hyperkeratosis, and sub-mucosal edema.

Conversely, no safety toxicity findings were observed after oral gavage administration of 5-LIO(Me-3,2-HOPO) once daily for 7 consecutive days to male rats at dose levels of 500, 1,000 or 2,000 $\mu\text{mol/kg/day}$ (203, 406 or 816 mg/kg/day). All animals survived to their scheduled sacrifice and clinical observations, body weights, hematology, serum clinical chemistry, gross observations at necropsy and histopathology examinations all appeared normal. Because toxicity was not observed at any of the 5-LIO(Me-3,2-HOPO) dose levels examined, the MTD could not be determined, and the no observed adverse effect level (NOAEL) is estimated to be $>2,000 \mu\text{mol/kg/day}$ (816 mg/kg/day), the highest dose level examined.

DISCUSSION

The preclinical development program for the hydroxypyridinonate chelators 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) is designed to meet regulatory criteria, as well as to provide sufficient animal efficacy and safety data for the determination of a presumed human safe and effective dose. Dose-response profiles in mice were established after ^{238}Pu or ^{241}Am contamination and subsequent parenteral or oral treatment with 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO) or Ca-DTPA. The results of these studies allow the determination of an optimal dose level for a specific route of treatment administration in mice: the minimum ligand dose levels that produced maximum decorporation efficacy of soluble ^{238}Pu were 1 $\mu\text{mol/kg}$ i.p. and 100 $\mu\text{mol/kg}$ po for 3,4,3-LI(1,2-HOPO) and 10 $\mu\text{mol/kg}$ i.p. and 500 $\mu\text{mol/kg}$ po for 5-LIO(Me-3,2-HOPO). The dose levels that produced maximum decorporation efficacy of soluble ^{241}Am were 60 $\mu\text{mol/kg}$ i.p. and 200 $\mu\text{mol/kg}$ po for 3,4,3-LI(1,2-HOPO) and 200 $\mu\text{mol/kg}$ i.p. and po for 5-LIO(Me-3,2-HOPO). The determination of a single adequate dose level for the simultaneous decorporation of a multitude of actinides is important because a large-scale contamination event may realistically involve several radionuclides. Thus, the finding that only slight differences exist in the decorporation efficacies of each ligand with two radionuclides, ^{238}Pu and ^{241}Am , is promising. While *in vivo* chelation mostly targets solubilized actinides in the circulatory system, there are some limitations to the decorporation models followed in our experiments, as the chosen contamination mode (i.v. injection of soluble citrate-actinide complexes) and treatment schedule (single treatment administered 1 h post-contamination) are unlikely to reflect a realistic scenario where masses of people may inhale insoluble actinide oxides and would not have access to such prompt treatment. To address these limitations, future bridging studies will be needed to test other modes of contamination such as inhalation, ingestion, or through wounds, as well as protracted and multiple treatment schedules.

The dose-response profile experiments also allow comparisons between the hydroxypyridinonate chelators and the existing treatment, Ca-DTPA. While accurate half maximal effective concentrations (EC_{50}) were not calculated, visual inspection of the nonlinear sigmoidal fits of the dose-dependent data show that in all cases the EC_{50} values for both hydroxypyridinonate chelators are substantially lower than for Ca-DTPA, with those of 3,4,3-LI(1,2-HOPO) being the lowest of the three. At equivalent dose levels, the experimental ligands are therefore considerably more effective than Ca-DTPA, independent of the route of administration.

The Animal Efficacy Rule Guidance document specifies that the pharmacokinetic/pharmacodynamic (PK/PD) relationship in contaminated animals must be investigated (14, 15). Such data is typically used to influence the doses selected for evaluation in human

volunteers during clinical trials. However, most radionuclide decorporation studies have followed an interspecies allometric scaling model because the stoichiometric ratio between the chelator and the targeted metal ion is critical to the mechanism of action. Allometric scaling is based on body weight and is consistent with the practice that is classically adopted in selecting 30 $\mu\text{mol/kg}$ Ca-DTPA or Zn-DTPA reference doses in animal efficacy studies. The currently recommended clinical dose of Ca-DTPA (1 g per 70 kg adult human, corresponding to a 30 $\mu\text{mol/kg}$ dose level) is based on historical decorporation results from different animal species (7–10). The allometric scaling approach is inconsistent with the FDA's PK/PD relationship requirement and also inconsistent with the accepted conversion system of animal doses into human equivalent doses (HED) based on body surface area (30). Following the HED body surface area conversion model, a 30 $\mu\text{mol/kg}$ Ca-DTPA human dose level corresponds to a 370 $\mu\text{mol/kg}$ mouse dose level. Because of these discrepancies, it seems important to compare the respective efficacies of 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) with both 30 and 370 $\mu\text{mol/kg}$ Ca-DTPA injection dose levels. We have thus chosen to express this as a horizontal line in all four panels of Fig. 1 to illustrate the amount of retained actinide that corresponds to a single parenteral 30 or 370 $\mu\text{mol/kg}$ dose of Ca-DTPA; the 370 $\mu\text{mol/kg}$ lines were obtained using the interpolated/extrapolated fits of the Ca-DTPA i.p. data. Such an analysis reveals that extremely high oral doses of Ca-DTPA would be needed to reach efficacious decorporation levels, independent of the targeted metal, while 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) can both reach the 370 $\mu\text{mol/kg}$ Ca-DTPA i.p. level when given orally at or below 200 $\mu\text{mol/kg}$. Following the body surface area conversion model, which we used to design the preclinical safety evaluations, a 200 $\mu\text{mol/kg}$ dose level in mice corresponds to a 16 $\mu\text{mol/kg}$ human dose level and a 100 $\mu\text{mol/kg}$ rat dose level.

A battery of *in vitro* and *in vivo* GLP genetic toxicology tests are necessary to establish the safety profiles of the new decorporation agents. The first two of three required genotoxicity tests, the bacterial reverse mutation Ames test and the *in vitro* mammalian cell chromosome aberration cytogenetic test, gave negative results indicating that both candidate ligands are nonmutagenic and nonclastogenic. The *in vivo* micronucleus test for chromosomal damage using rodent hematopoietic cells will be conducted in the near future. No further genotoxicity tests will be warranted if the results of this *in vivo* test also turns out to be negative (22). Even if the results of the *in vivo* micronucleus test is positive with either ligand, the threshold dose level below which chromosome loss or breakage is not expected can be determined.

Previous GLP-compliant subchronic toxicity studies demonstrated that both 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) are well tolerated in male and female Sprague-Dawley rats at the respective doses of 102 $\mu\text{mol/kg}$ and 157 $\mu\text{mol/kg}$, following 28 days of once daily oral administration. Using the body surface scaling methods described above, a dose of 100 $\mu\text{mol/kg}$ of both ligands is expected to be safe, well-tolerated and efficacious in rats (12). In the MTD studies described above, 5-LIO(Me-3,2-HOPO) was administered orally to male Sprague-Dawley rats, at doses up to 2,000 $\mu\text{mol/kg/day}$ (816 mg/kg/day) for 7 consecutive days, which resulted in no signs of toxicity. Although 5-LIO(Me-3,2-HOPO) was well-tolerated at levels 10-fold the presumed efficacious oral dose, these ligands have poor oral bioavailability in rats (<5%) (17, 31), and this is an important consideration for future safety and efficacy tests with 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) in other species. Conversely, the MTD for 3,4,3-LI(1,2-HOPO) in male rats is lower than 532 $\mu\text{mol/kg/day}$ (400 mg/kg/day) based on histopathologic changes observed in the glandular and nonglandular stomach. The previous 28-day repeat dose GLP studies examined 3,4,3-LI(1,2-HOPO) at levels as high as 102 $\mu\text{mol/kg/day}$ in male and female Sprague-Dawley rats (12), and no dose-limiting toxicities were observed. We therefore estimate that the repeat dose MTD for 3,4,3-LI(1,2-HOPO) in rats should be between 102 $\mu\text{mol/kg/day}$ to 532 $\mu\text{mol/kg/day}$.

day, which is one to five times above the presumed target oral efficacy dose calculated from the single dose efficacy studies described above.

The hydroxypyridinonate ligands 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) are under development for the treatment of individuals with known or suspected contamination with Pu, Am, Cm, U or Np to increase the rates of elimination of these radionuclides. While a number of additional efficacy and preclinical safety studies must be performed before early phase clinical trials can be conducted, the studies reported here provide additional support to the promising efficacy and safety profiles of both compounds. The dose-response profiles obtained for parenteral and oral treatment with 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) in mice after intravenous ^{238}Pu and ^{241}Am contamination confirm substantial decorporation improvements over the existing DTPA-based human chelation options even when two different interspecies scaling methods are taken into consideration. The oral doses at which maximum decorporation efficacy was obtained with both ligands in mice were demonstrated safe in rats after repeated daily administration over 7 to 28 days. MTD levels in rats for daily administration over 7 days were found one to fivefold higher than the presumed single efficacious dose for 3,4,3-LI(1,2-HOPO) and could not be determined for 5-LIO(Me-3,2-HOPO), as doses 10-fold higher than the presumed single efficacious 5-LIO(Me-3,2-HOPO) dose did not cause any observable toxicity. Finally, a battery of genotoxicity tests demonstrated that both chelators are nongenotoxic. Plans for further efficacy testing with different relevant isotopes, routes of contamination and animal species are underway. These results will be coupled with additional safety toxicology tests to determine whether only one or both decorporation agents will be selected for further development and to predict an optimal human dosing regimen for initial clinical investigations.

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REFERENCES

1. Katz, J.J.; Seaborg, G.T.; Morss, L.R. The chemistry of the actinide elements. 2nd ed.. Chapman and Hall; London: 1986.
2. Taylor DM, Stradling GN, Henge-Napoli MH. The scientific background to decorporation. *Radiat Prot Dosim.* 2000; 87:11–7.
3. Paquet F, Frelon S, Cote G, Madic C. The contribution of chemical speciation to internal dosimetry. *Radiat Prot Dosim.* 2003; 105:179–85.
4. Durbin PW, Lauriston S. Taylor Lecture: The quest for therapeutic actinide chelators. *Health Phys.* 2008; 95:465–92. [PubMed: 18849679]
5. Gorden AEV, Xu J, Raymond KN, Durbin PW. The rational design of sequestering agents for plutonium and other actinide elements. *Chem Rev.* 2003; 103:4207–82. [PubMed: 14611263]
6. Guidance for industry: Calcium DTPA and zinc DTPA drug products - submitting a new drug application. U.S. Food and Drug Administration; Silver Spring: 2004.
7. Breitenstein BD, Palmer HE. Life-time follow-up of the 1976 americium accident victim. *Radiat Prot Dosim.* 1989; 26:317–22.
8. Durbin, PW. Actinides in animals and man. In: Morss, L.R.; Edelstein, N.M.; Fuger, J., editors. The chemistry of the actinide and transactinide elements. Springer; Netherlands: 2006. p. 3339–440.

9. NCRP Report No. 161. National Council on Radiation Protection and Measurements; Bethesda: 2008. Management of persons contaminated with radionuclides: Handbook.
10. Stradling GN, Henge-Napoli MH, Paquet F, Poncy J-L, Fritsch P, Taylor DM. Optimum treatment regimens with animals. *Radiat Prot Dosim.* 2000; 87:29–40.
11. REAC/TS Newsletter. Radiation Emergency Assistance Center/Training Site; Oak Ridge: 1981. Update on chelation therapy: Chelators should not be used for uranium or neptunium.
12. Abergel RJ, Durbin PW, Kullgren B, Ebbe SN, Xu J, Chang PY, et al. Biomimetic actinide chelators: An update on the preclinical development of the orally active hydroxypyridonate decorporation agents 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO). *Health Phys.* 2010; 99:401–7. [PubMed: 20699704]
13. Abergel RJ, Raymond KN. Multidentate terephthalamidate and hydroxypyridonate ligands: Towards new orally active chelators. *Hemoglobin.* 2011; 35:276–90. [PubMed: 21599440]
14. Guidance for industry: Internal radioactive contamination - development of decorporation agents. U.S. Food and Drug Administration; Silver Spring: 2006.
15. Guidance for industry: Animal models - essential elements to address efficacy under the animal rule (draft guidance). U.S. Food and Drug Administration; Silver Spring: 2009.
16. Code of federal regulations title 21, pt. 314. U.S. Food and Drug Administration; Silver Spring: 2011. Approval of new drugs when human efficacy studies are not ethical or feasible.
17. Chang PY, Bunin DI, Gow J, Swezey R, Shinn W, Shuh DK, et al. Analytical methods for the bioavailability evaluation of hydroxypyridinonate actinide decorporation agents in pre-clinical pharmacokinetic studies. *J Chromatograph Separat Techniq.* 2012; S4
18. Code of federal regulations title 9, pt. 3. Animal and Plant Health Inspection Service; Washington: 2012. Animals and animal products - standards.
19. Durbin PW, Kullgren B, Xu J, Raymond KN. In vivo chelation of Am(III), Pu(IV), Np(V), and U(VI) in mice by tren(Me-3,2-HOPO). *Radiat Prot Dosim.* 1994; 53:305–9.
20. Durbin PW, Kullgren B, Xu J, Raymond KN. Multidentate hydroxypyridinonate ligands for Pu(IV) chelation in vivo: Comparative efficacy and toxicity in mouse of ligands containing 1,2-HOPO or Me-3,2-HOPO. *Int J Radiat Biol.* 2000; 76:199–214. [PubMed: 10716641]
21. Cimino MC. Comparative overview of current international strategies and guidelines for genetic toxicology testing for regulatory purposes. *Environ Mol Mutagen.* 2006; 47:362–90. [PubMed: 16649190]
22. Step 4 version of the ICH process. International Conference on Harmonization; Geneva: 2011. ICH harmonized tripartite guideline S2(R1): Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use.
23. Code of federal regulations title 21, pt. 58. U.S. Food and Drug Administration; Silver Spring: 2010. Good laboratory practice for nonclinical laboratory studies.
24. Doppalapudi RS, Riccio ES, Davis Z, Menda S, Wang A, Du N, et al. Genotoxicity of the cancer chemopreventive drug candidates. *Mutat Res.* 2012; 746:78–88. [PubMed: 22498038]
25. Mortelmans KE, Riccio ES. The bacterial tryptophan reverse mutation assay with *Escherichia coli* WP2. *Mutat Res.* 2000; 455:61–9. [PubMed: 11113467]
26. Mortelmans KE, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. *Mutat Res.* 2000; 455:29–60. [PubMed: 11113466]
27. Galloway SM, Bloom AD, Resnick M, Margolin BH, Nakamura F, Archer P, et al. Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. *Environ Mutagen.* 1985; 7:1–51. [PubMed: 3967632]
28. Savage JR. Classification and relationships of induced chromosomal structural changes. *J Med Genet.* 1976; 13:103–22. [PubMed: 933108]
29. Xu J, Durbin PW, Kullgren B, Ebbe SN, Uhlir LC, Raymond KN. Synthesis and initial evaluation for in vivo chelation of Pu(IV) of a mixed octadentate spermine-based ligand containing 4-carbamoyl-3-hydroxy-1-methyl-2(1H)-pyridinone and 6-carbamoyl-1-hydroxy-2(1H)-pyridinone. *J Med Chem.* 2002; 45:3963–71. [PubMed: 12190318]
30. Guidance for industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. U.S. Food and Drug Administration; Silver Spring: 2005.

31. Durbin PW, Kullgren B, Xu J, Raymond KN, Henge-Napoli MH, Bailly T, et al. Octadentate hydroxypyridinonate (HOPO) ligands for plutonium (IV): Pharmacokinetics and oral efficacy. *Radiat Prot Dosim.* 2003; 105:503–8.

FIG. 1.

Dose-dependent total body retention of ^{238}Pu and ^{241}Am after parenteral or oral treatment with 3,4,3-LI(1,2-HOPO), 5-LIO(Me-3,2-HOPO) or Ca-DTPA. Ligands were given to groups of five mice by i.p. injection (panels A and C) or oral gavage (panels B and D) 1 h after i.v. injection of ^{238}Pu -citrate (panels A and B) or ^{241}Am -citrate (panels C and D). Mice were euthanized 24 h after contamination. Previously reported data was used in panel A (20, 29). Data, expressed as percentage of injected radionuclide (%ID, mean $\pm$ SD), were normalized to 100% material recovery for each five-mouse group. Each five-mouse group is represented by a single data point, while lines are sigmoidal logistic fits of the corresponding dose-response profile. Horizontal lines represent the amount of retained actinide corresponding to a single parenteral Ca-DTPA clinical dose using body weight (30 $\mu\text{mol/kg}$) or body surface area (370 $\mu\text{mol/kg}$) interspecies scaling.

FIG. 2.

Cytogenetic evaluation of cells exposed to 3,4,3-LI(1,2-HOPO). Assayed at 62.5, 125, 250 and 500 µg/ml, 3,4,3-LI(1,2-HOPO) did not induce chromosomal abnormalities in the CHO assay. Only the positive control cultures had a statistically significant increase in the number of cells with structural aberrations (38 to 42%) compared to the respective solvent control group ($P < 0.05$). Cells were cultured for 3 or 21 h without S9 (–) or for 3 h with S9 (+).

FIG. 3.

Cytogenetic evaluation of cells exposed to 5-LIO(Me-3,2-HOPO). Assayed at 100, 200 and 400 µg/ml, 5-LIO(Me-3,2-HOPO) did not induce chromosomal abnormalities in the CHO assay. Only the positive control cultures had a statistically significant increase in the number of cells with structural aberrations (26.4 to 30%) compared to the respective solvent control group ($P < 0.05$). Cells were cultured for 3 or 21 h without S9 (–) or for 3 h with S9 (+).

FIG. 4.

Oral administration of 3,4,3-LI(1,2-HOPO) for 7 days to male Sprague-Dawley rats resulted in dose-dependent decreases in (panel A) the absolute number of reticulocytes and (panel B) the number of reticulocytes as a percentage of the total red blood cell (RBC) population. Mean group values $\pm$ standard deviation were evaluated using one-way ANOVA followed by Dunnett's test. *Significant difference from control ($P < 0.05$). **Significant difference from control ($P < 0.01$).

TABLE 1

Dose-Dependent Removal of ^{238}Pu from Mice by Orally Administered Hydroxypyridinonate Ligands and Ca-DTPA^a

Treatment	Dose ($\mu\text{mol/kg}$)	Percentage of injected $^{238}\text{Pu} \pm \text{SD at } 24 \text{ h}^b$					
		Tissues				Excreta ^c	
		Skeleton	Liver	Soft tissue	Kidneys	Urine	Feces and GI content
3,4,3-LI(1,2-HOPO), po	1	31 $\pm$ 3.4	40 $\pm$ 4.3	3.7 $\pm$ 0.8 ^d	0.7 $\pm$ 0.2 ^d	9.0	16
	3	29 $\pm$ 4.1	41 $\pm$ 4.0	3.8 $\pm$ 0.1 ^d	0.9 $\pm$ 0.1	9.9	16
	10	29 $\pm$ 7.4	30 $\pm$ 6.5 ^d	3.4 $\pm$ 0.6 ^d	0.8 $\pm$ 0.2	13	23
	30 ^e	21 $\pm$ 6.6 ^d	19 $\pm$ 5.8 ^d	3.1 $\pm$ 1.4 ^d	0.6 $\pm$ 0.3 ^d	18	43
	60	20 $\pm$ 2.1 ^d	19 $\pm$ 5.8 ^d	2.2 $\pm$ 0.6 ^d	0.4 $\pm$ 0.1 ^d	18	40
	100 ^e	13 $\pm$ 1.4 ^d	5.7 $\pm$ 3.4 ^d	3.0 $\pm$ 1.2 ^d	0.5 $\pm$ 0.2 ^d	19	60
	200	15 $\pm$ 2.4 ^d	3.9 $\pm$ 1.6 ^d	1.1 $\pm$ 0.2 ^d	0.3 $\pm$ 0.1 ^d	23	57
5-LIO(Me-3,2-HOPO), po	3	34 $\pm$ 4.3	45 $\pm$ 5.8	3.2 $\pm$ 0.8 ^d	1.2 $\pm$ 0.3	6.6	10
	10	38 $\pm$ 3.1	40 $\pm$ 2.3	3.1 $\pm$ 0.9 ^d	1.1 $\pm$ 0.1	7.2	11
	30	33 $\pm$ 6.2	39 $\pm$ 8.6	2.8 $\pm$ 1.0 ^d	0.9 $\pm$ 0.2	7.5	18
	60	33 $\pm$ 4.2	29 $\pm$ 3.3 ^d	3.9 $\pm$ 0.5	0.9 $\pm$ 0.1	9.1	24
	100 ^e	30 $\pm$ 6.5	14 $\pm$ 6.2 ^d	4.6 $\pm$ 0.8	0.7 $\pm$ 0.3 ^d	8.1	42
	200 ^e	20 $\pm$ 2.6 ^d	5.7 $\pm$ 1.7 ^d	3.6 $\pm$ 0.3 ^d	0.5 $\pm$ 0.1 ^d	14	57
	500	17 $\pm$ 0.8 ^d	2.9 $\pm$ 0.8 ^d	1.6 $\pm$ 0.3 ^d	0.3 $\pm$ 0.1 ^d	12	67
Ca-DTPA, po	3	29 $\pm$ 4.2	46 $\pm$ 4.3	4.3 $\pm$ 0.6	1.3 $\pm$ 0.4	8.1	11
	10	27 $\pm$ 4.4	49 $\pm$ 3.2	5.0 $\pm$ 1.0	1.3 $\pm$ 0.3	8.2	10
	30	37 $\pm$ 5.9	38 $\pm$ 6.3	3.5 $\pm$ 0.9 ^d	1.0 $\pm$ 0.2	9.6	11
	60	32 $\pm$ 4.0	40 $\pm$ 2.4	3.5 $\pm$ 1.4 ^d	1.2 $\pm$ 0.1	12	11
	100 ^e	28 $\pm$ 3.5	40 $\pm$ 3.5	6.4 $\pm$ 0.9	1.2 $\pm$ 0.3	19	5.8
	500	26 $\pm$ 4.6	30 $\pm$ 1.8 ^d	3.4 $\pm$ 1.1 ^d	0.7 $\pm$ 0.2 ^d	30	11
Controls, po		31 $\pm$ 5.3	44 $\pm$ 7.1	5.6 $\pm$ 0.6	1.2 $\pm$ 0.2	8.5	9.9

^aLigands were given orally to groups of five mice at 1 h after i.v. injection of Pu-citrate (0.2 ml, 0.74 kBq), and mice were euthanized at 24 h.

^bData, expressed as percentage of injected ^{238}Pu (%ID, mean $\pm$ SD, 5 mice per group), were normalized to 100% material recovery for each five-mouse group. Discrepancies are due to rounding.

^cExcreta of each five-mouse group were pooled and SD is not available.

^dMean is significantly less than for corresponding controls ($P < 0.01$, 1-way ANOVA followed by post hoc analysis with Dunnett's multiple comparison test).

^eData previously reported and discussed (12, 13).

TABLE 2

Dose-Dependent Removal of ^{241}Am from Mice by Injected Hydroxypyridinonate Ligands and Ca-DTPA^a

Treatment	Dose ($\mu\text{mol/kg}$)	Percentage of injected $^{241}\text{Am} \pm \text{SD}$ at 24 h ^b					
		Tissues				Excreta ^c	
		Skeleton	Liver	Soft tissue	Kidneys	Urine	Feces and GI content
3,4,3-LI(1,2-HOPO), i.p.	0.3	16 $\pm$ 0.8 ^d	14 $\pm$ 2.7 ^d	4.4 $\pm$ 0.9	0.6 $\pm$ 0.1 ^d	15	50
	1	18 $\pm$ 2.1 ^d	7.0 $\pm$ 1.3 ^d	4.7 $\pm$ 2.5	0.5 $\pm$ 0.1 ^d	21	49
	3	18 $\pm$ 1.4 ^d	5.3 $\pm$ 1.8 ^d	3.2 $\pm$ 0.3 ^d	0.4 $\pm$ 0.1 ^d	19	55
	10	17 $\pm$ 1.1 ^d	3.9 $\pm$ 0.9 ^d	3.0 $\pm$ 1.0 ^d	0.5 $\pm$ 0.1 ^d	20	56
	30 ^e	12 $\pm$ 1.0 ^d	3.2 $\pm$ 1.0 ^d	4.6 $\pm$ 0.8	0.3 $\pm$ 0.1 ^d	17	62
	60	10 $\pm$ 0.9 ^d	2.8 $\pm$ 0.9 ^d	2.2 $\pm$ 0.4 ^d	0.9 $\pm$ 0.3	24	60
	100	7.6 $\pm$ 0.6 ^d	2.2 $\pm$ 0.4 ^d	2.2 $\pm$ 0.4 ^d	0.7 $\pm$ 0.1	25	63
	200	7.5 $\pm$ 1.1 ^d	6.7 $\pm$ 1.9 ^d	1.9 $\pm$ 0.3 ^d	0.8 $\pm$ 0.1	25	58
5-LIO(Me-3,2-HOPO), i.p.	1	18 $\pm$ 2.1	15 $\pm$ 2.4 ^d	4.8 $\pm$ 0.4	0.7 $\pm$ 0.2 ^d	15	46
	3	22 $\pm$ 1.4	8.9 $\pm$ 2.2 ^d	3.6 $\pm$ 0.2	0.4 $\pm$ 0.1 ^d	20	46
	10	21 $\pm$ 2.2	5.6 $\pm$ 0.7 ^d	3.4 $\pm$ 0.7	0.3 $\pm$ 0.1 ^d	19	51
	30	18 $\pm$ 1.3 ^d	5.1 $\pm$ 0.6 ^d	3.6 $\pm$ 1.8	0.3 $\pm$ 0.1 ^d	22	51
	60	16 $\pm$ 2.0 ^d	3.8 $\pm$ 0.7 ^d	3.2 $\pm$ 0.5	0.3 $\pm$ 0.1 ^d	17	60
	100 ^e	15 $\pm$ 0.4 ^d	4.0 $\pm$ 0.5 ^d	4.5 $\pm$ 0.7	0.3 $\pm$ 0.1 ^d	21	55
	200	14 $\pm$ 2.5 ^d	4.8 $\pm$ 1.1 ^d	3.4 $\pm$ 0.5	0.4 $\pm$ 0.1 ^d	21	57
	500	11 $\pm$ 1.3 ^d	3.8 $\pm$ 0.4 ^d	6.3 $\pm$ 0.9	0.3 $\pm$ 0.1 ^d	25	54
Ca-DTPA, i.p.	1	22 $\pm$ 0.9	54 $\pm$ 2.7	5.9 $\pm$ 0.6	0.7 $\pm$ 0.1 ^d	16	2.2
	3	23 $\pm$ 1.7	42 $\pm$ 3.5 ^d	5.5 $\pm$ 0.8	0.6 $\pm$ 0.1 ^d	26	2.8
	10	23 $\pm$ 2.2	40 $\pm$ 6.3 ^d	5.3 $\pm$ 0.8	0.5 $\pm$ 0.1 ^d	26	4.7
	30	21 $\pm$ 4.3	33 $\pm$ 7.0 ^d	4.5 $\pm$ 1.1	0.6 $\pm$ 0.1 ^d	34	7.0
	60	19 $\pm$ 2.1 ^d	22 $\pm$ 1.3 ^d	6.4 $\pm$ 0.7	0.4 $\pm$ 0.1 ^d	36	16
	200	15 $\pm$ 2.3 ^d	15 $\pm$ 3.3 ^d	4.1 $\pm$ 0.9	0.5 $\pm$ 0.1 ^d	38	27
	500	15 $\pm$ 0.8 ^d	9.7 $\pm$ 0.8 ^d	4.3 $\pm$ 1.2	0.4 $\pm$ 0.1 ^d	45	25
Controls, i.p.		24 $\pm$ 2.6	54 $\pm$ 3.8	6.1 $\pm$ 0.7	1.0 $\pm$ 0.3	13	1.8

^aLigands were given by i.p. injection to groups of five mice at 1 h after i.v. injection of Am-citrate (0.2 ml, 0.43 kBq), and mice were euthanized at 24 h.

^bData, expressed as percent of injected ^{241}Am (% ID, mean $\pm$ SD, 5 mice per group), were normalized to 100% material recovery for each five-mouse group. Discrepancies are due to rounding.

^cExcreta of each five-mouse group were pooled and SD is not available.

^d Mean is significantly less than for corresponding controls ($P < 0.01$, 1-way ANOVA followed by post hoc analysis with Dunnett's multiple comparison test).

^e Data previously reported and discussed (12, 13).

TABLE 3

Dose-Dependent Removal of ^{241}Am from Mice by Orally Administered Hydroxypyridinonate Ligands and Ca-DTPA^a

Treatment	Dose ($\mu\text{mol/kg}$)	Percentage of injected $^{241}\text{Am} \pm \text{SD}$ at 24 h ^b					
		Tissues				Excreta ^c	
		Skeleton	Liver	Soft tissue	Kidneys	Urine	Feces and GI content
3,4,3-LI(1,2-HOPO), po	1	29 $\pm$ 4.0	46 $\pm$ 5.4	5.9 $\pm$ 1.3	0.8 $\pm$ 0.3	16	2.3
	3	28 $\pm$ 1.8	47 $\pm$ 5.1	5.0 $\pm$ 0.8	0.7 $\pm$ 0.1	14	6.1
	10	28 $\pm$ 1.4 ^d	47 $\pm$ 3.6	4.9 $\pm$ 0.8	0.7 $\pm$ 0.2	14	5.2
	30 ^e	23 $\pm$ 1.2	37 $\pm$ 8.7 ^d	4.2 $\pm$ 0.6	0.6 $\pm$ 0.1	14	21
	60	26 $\pm$ 2.5	30 $\pm$ 5.9 ^d	4.3 $\pm$ 0.4	0.9 $\pm$ 0.2	16	23
	100 ^e	21 $\pm$ 0.9 ^d	17 $\pm$ 7.1 ^d	4.2 $\pm$ 0.4	0.5 $\pm$ 0.1 ^d	16	42
	200	20 $\pm$ 1.7 ^d	5.9 $\pm$ 2.2 ^d	3.1 $\pm$ 0.6 ^d	1.1 $\pm$ 0.2	17	53
5-LIO(Me-3,2-HOPO), po	3	30 $\pm$ 2.0	50 $\pm$ 1.7	4.8 $\pm$ 0.6	0.8 $\pm$ 0.1	11	3.5
	10	28 $\pm$ 2.2	48 $\pm$ 2.1	5.0 $\pm$ 0.8	0.7 $\pm$ 0.1	15	3.9
	30	30 $\pm$ 3.5	43 $\pm$ 4.6 ^d	4.7 $\pm$ 0.9	0.8 $\pm$ 0.2	13	8.2
	60	28 $\pm$ 3.3	35 $\pm$ 4.8 ^d	5.8 $\pm$ 0.5	0.8 $\pm$ 0.1	12	18
	100 ^e	24 $\pm$ 2.1	15 $\pm$ 4.4 ^d	5.0 $\pm$ 0.5	0.6 $\pm$ 0.1	15	41
	200 ^e	22 $\pm$ 2.1	7.2 $\pm$ 1.4 ^d	4.7 $\pm$ 0.3	0.5 $\pm$ 0.1 ^d	15	51
	500	27 $\pm$ 2.3	5.5 $\pm$ 0.6 ^d	4.4 $\pm$ 0.5	0.7 $\pm$ 0.4	13	49
Ca-DTPA, po	3	30 $\pm$ 2.4	49 $\pm$ 3.3	5.5 $\pm$ 0.8	0.9 $\pm$ 0.2	13	2.0
	10	30 $\pm$ 2.7	48 $\pm$ 4.7	5.0 $\pm$ 0.5	0.8 $\pm$ 0.2	14	2.5
	30	26 $\pm$ 1.4	49 $\pm$ 4.0	5.0 $\pm$ 0.5	0.8 $\pm$ 0.1	16	4.0
	60	26 $\pm$ 2.8	44 $\pm$ 4.5 ^d	5.5 $\pm$ 0.7	0.5 $\pm$ 0.2 ^d	20	4.2
	100 ^e	22 $\pm$ 2.0 ^d	49 $\pm$ 3.6	5.0 $\pm$ 0.3	0.7 $\pm$ 0.1	19	4.2
	500	25 $\pm$ 3.7	39 $\pm$ 5.1 ^d	4.6 $\pm$ 0.6	0.5 $\pm$ 0.1 ^d	26	5.2
Controls, po		28 $\pm$ 0.9	57 $\pm$ 0.2	4.9 $\pm$ 0.3	1.0 $\pm$ 0.2	7.9	1.7

^aLigands were given orally to groups of five mice at 1 h after i.v. injection of Am-citrate (0.2 ml, 0.65 kBq), and mice were euthanized at 24 h.

^bData, expressed as percentage of injected ^{241}Am (%ID, mean $\pm$ SD, 5 mice per group), were normalized to 100% material recovery for each five-mouse group. Discrepancies are due to rounding.

^cExcreta of each five-mouse group were pooled and SD is not available.

^dMean is significantly less than for corresponding controls ($P < 0.01$, 1-way ANOVA followed by post hoc analysis with Dunnett's multiple comparison test).

^eData previously reported and discussed (12, 13).

TABLE 4

Analysis of 3,4,3-LI(1,2-HOPO) Mutagenicity in Bacterial Strains of the Ames Test

Dose ($\mu\text{g}/\text{plate}$)	Mean $\pm$ SD revertant colonies/plate									
	TA1535	TA1537	TA98	TA100	WP2uvrA	TA1535	TA1537	TA98	TA100	WP2uvrA
Without S9										
0	17 $\pm$ 3	16 $\pm$ 3	39 $\pm$ 64	168 $\pm$ 13	32 $\pm$ 4	13 $\pm$ 3	17 $\pm$ 4	37 $\pm$ 5	165 $\pm$ 18	41 $\pm$ 8
156.3	18 $\pm$ 1	14 $\pm$ 2	32 $\pm$ 4	166 $\pm$ 9	34 $\pm$ 1	17 $\pm$ 2	14 $\pm$ 2	40 $\pm$ 4	165 $\pm$ 11	37 $\pm$ 3
312.5	14 $\pm$ 4	11 $\pm$ 3	35 $\pm$ 4	151 $\pm$ 18	27 $\pm$ 4	16 $\pm$ 4	12 $\pm$ 2	34 $\pm$ 3	160 $\pm$ 4	29 $\pm$ 4
625	14 $\pm$ 2	11 $\pm$ 1	35 $\pm$ 4	143 $\pm$ 5	18 $\pm$ 5	17 $\pm$ 1	12 $\pm$ 1	22 $\pm$ 16	161 $\pm$ 10	20 $\pm$ 5
1,250	13 $\pm$ 1	10 $\pm$ 1	28 $\pm$ 2	154 $\pm$ 3	cytotoxic	14 $\pm$ 1	11 $\pm$ 3	41 $\pm$ 1	160 $\pm$ 4	cytotoxic
2,500	18 $\pm$ 2	8 $\pm$ 2	30 $\pm$ 1	155 $\pm$ 3	cytotoxic	14 $\pm$ 2	9 $\pm$ 2	42 $\pm$ 2	149 $\pm$ 3	cytotoxic
5,000	14 $\pm$ 2	9 $\pm$ 1	34 $\pm$ 3	134 $\pm$ 13	cytotoxic	15 $\pm$ 3	7 $\pm$ 2	36 $\pm$ 2	131 $\pm$ 4	cytotoxic
With 5% S9										
0	19 $\pm$ 2	25 $\pm$ 6	19 $\pm$ 5	183 $\pm$ 10	29 $\pm$ 3	15 $\pm$ 9	17 $\pm$ 6	43 $\pm$ 6	193 $\pm$ 10	36 $\pm$ 5
39.1	NT	NT	NT	NT	28 $\pm$ 8	NT	NT	NT	NT	31 $\pm$ 5
78.1	NT	NT	NT	NT	26 $\pm$ 3	NT	NT	NT	NT	37 $\pm$ 4
156.3	24 $\pm$ 12	14 $\pm$ 2	26 $\pm$ 1	244 $\pm$ 7*	28 $\pm$ 2	9 $\pm$ 2	21 $\pm$ 2	42 $\pm$ 8	175 $\pm$ 15	32 $\pm$ 4
312.5	18 $\pm$ 2	15 $\pm$ 1	34 $\pm$ 2*	214 $\pm$ 8	25 $\pm$ 2	16 $\pm$ 5	16 $\pm$ 5	41 $\pm$ 8	175 $\pm$ 23	29 $\pm$ 3
625	18 $\pm$ 2	11 $\pm$ 4	28 $\pm$ 4	211 $\pm$ 18	cytotoxic	13 $\pm$ 3	22 $\pm$ 1	38 $\pm$ 4	201 $\pm$ 26	cytotoxic
1,250	11 $\pm$ 1	14 $\pm$ 4	36 $\pm$ 5*	179 $\pm$ 9	cytotoxic	17 $\pm$ 4	12 $\pm$ 2	36 $\pm$ 3	182 $\pm$ 6	cytotoxic
2,500	18 $\pm$ 4	16 $\pm$ 6	31 $\pm$ 3*	193 $\pm$ 5	cytotoxic	11 $\pm$ 5	9 $\pm$ 2	46 $\pm$ 5	193 $\pm$ 11	cytotoxic
5,000	26 $\pm$ 4	14 $\pm$ 2	33 $\pm$ 1*	194 $\pm$ 16	NT	20 $\pm$ 2	10 $\pm$ 2	48 $\pm$ 1	186 $\pm$ 10	NT
With 10% S9										
0	19 $\pm$ 2	25 $\pm$ 6	19 $\pm$ 5	183 $\pm$ 10	29 $\pm$ 3	15 $\pm$ 9	17 $\pm$ 6	43 $\pm$ 6	193 $\pm$ 10	36 $\pm$ 5
39.1	NT	NT	NT	NT	28 $\pm$ 8	NT	NT	NT	NT	31 $\pm$ 5
78.1	NT	NT	NT	NT	26 $\pm$ 3	NT	NT	NT	NT	37 $\pm$ 4
156.3	24 $\pm$ 12	14 $\pm$ 2	26 $\pm$ 1	244 $\pm$ 7*	28 $\pm$ 2	9 $\pm$ 2	21 $\pm$ 2	42 $\pm$ 8	175 $\pm$ 15	32 $\pm$ 4
312.5	18 $\pm$ 2	15 $\pm$ 1	34 $\pm$ 2*	214 $\pm$ 8	25 $\pm$ 2	16 $\pm$ 5	16 $\pm$ 5	41 $\pm$ 8	175 $\pm$ 23	29 $\pm$ 3
625	18 $\pm$ 2	11 $\pm$ 4	28 $\pm$ 4	211 $\pm$ 18	cytotoxic	13 $\pm$ 3	22 $\pm$ 1	38 $\pm$ 4	201 $\pm$ 26	cytotoxic
1,250	11 $\pm$ 1	14 $\pm$ 4	36 $\pm$ 5*	179 $\pm$ 9	cytotoxic	17 $\pm$ 4	12 $\pm$ 2	36 $\pm$ 3	182 $\pm$ 6	cytotoxic
2,500	18 $\pm$ 4	16 $\pm$ 6	31 $\pm$ 3*	193 $\pm$ 5	cytotoxic	11 $\pm$ 5	9 $\pm$ 2	46 $\pm$ 5	193 $\pm$ 11	cytotoxic
5,000	26 $\pm$ 4	14 $\pm$ 2	33 $\pm$ 1*	194 $\pm$ 16	NT	20 $\pm$ 2	10 $\pm$ 2	48 $\pm$ 1	186 $\pm$ 10	NT

* Significant ($P < 0.01$) by Dunnett's test. NT = not tested.

TABLE 5
Analysis of 5-LIO(Me-3,2-HOPO) Mutagenicity in Bacterial Strains of the Ames Test

Dose (µg/plate)	Mean ± SD Revertant Colonies/Plate									
	TA1535	TA1537	TA98	TA100	WP2uvrA	TA1535	TA1537	TA98	TA100	WP2uvrA
	Without S9					With 5% S9				
0	13 ± 2	15 ± 3	35 ± 4	124 ± 3	39 ± 4	14 ± 3	17 ± 2	37 ± 6	145 ± 6	42 ± 2
156.3	16 ± 2	11 ± 2	28 ± 2	119 ± 1	28 ± 4	8 ± 2	12 ± 2	42 ± 6	133 ± 3	24 ± 3
312.5	14 ± 2	12 ± 0	22 ± 2	117 ± 2	19 ± 1	14 ± 3	10 ± 2	31 ± 5	132 ± 4	27 ± 3
625	15 ± 1	10 ± 1	24 ± 2	131 ± 2	26 ± 4	16 ± 1	7 ± 2	31 ± 5	134 ± 4	26 ± 1
1250	17 ± 2	17 ± 2	40 ± 5	140 ± 3*	cytotoxic	12 ± 1	4 ± 2	51 ± 6*	132 ± 3	23 ± 3
2500	5 ± 2	7 ± 1	44 ± 6	141 ± 3*	cytotoxic	12 ± 2	9 ± 1	36 ± 4	135 ± 3	12 ± 2
5000	14 ± 4	17 ± 1	58 ± 7*	170 ± 7*	cytotoxic	12 ± 2	12 ± 2	51 ± 4*	160 ± 6*	cytotoxic
	Without S9					With 10% S9				
0	15 ± 3	12 ± 3	31 ± 3	146 ± 9	30 ± 4	16 ± 2	15 ± 2	34 ± 7	158 ± 24	43 ± 4
39.1	NT	NT	NT	NT	31 ± 4	NT	NT	NT	NT	51 ± 5
78.1	NT	NT	NT	NT	20 ± 0	NT	NT	NT	NT	41 ± 1
156.3	18 ± 6	13 ± 2	31 ± 4	153 ± 7	31 ± 5	13 ± 3	15 ± 0	38 ± 3	141 ± 2	34 ± 6
312.5	12 ± 3	12 ± 2	25 ± 2	157 ± 3	25 ± 2	13 ± 2	13 ± 1	40 ± 4	141 ± 7	26 ± 3
625	16 ± 3	12 ± 3	36 ± 2	155 ± 12	25 ± 4	13 ± 2	10 ± 3	43 ± 2	115 ± 11	37 ± 2
1250	12 ± 3	14 ± 2	27 ± 4	137 ± 6	15 ± 4	12 ± 2	11 ± 1	39 ± 3	170 ± 3	18 ± 6
2500	16 ± 4	8 ± 3	42 ± 7*	148 ± 1	cytotoxic	8 ± 1	11 ± 1	42 ± 6	143 ± 2	cytotoxic
5000	14 ± 3	5 ± 0	57 ± 2*	146 ± 9	NT	14 ± 2	9 ± 2	45 ± 3	153 ± 1	NT

* Significant ($P < 0.01$) by Dunnett's test. NT = not tested.