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Orally administered DTPA penta-ethyl ester for the decorporation of inhaled ^{241}Am

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Abstract

Diethylenetriaminepentaacetic acid (DTPA) is an effective decorporation agent to facilitate the elimination of radionuclides from the body, but its permeability-limited oral bioavailability limits its utility in mass-casualty emergencies. To overcome this limitation, a prodrug strategy using the penta-ethyl ester form of DTPA is under investigation. Pharmacokinetic and biodistribution studies were conducted in rats by orally administering [^{14}C]DTPA penta-ethyl ester, and this prodrug and its hydrolysis products were analyzed as a single entity. Compared to a previous reporting of intravenously administered DTPA, the oral administration of this prodrug resulted in a sustained plasma concentration profile with higher plasma exposure and lower clearance. An assessment of the urine composition revealed that the bioactivation was extensive but incomplete, with no detectable levels of the penta- or tetra-ester forms. Tissue distribution at 12 h was limited, with approximately 73% of the administered dose being associated with the gastrointestinal tract. In the efficacy study, rats were exposed to aerosols of ^{241}Am nitrate before receiving a single oral treatment of the prodrug. The urinary excretion of ^{241}Am was found to be 19% higher than with the control. Consistent with prior reports of DTPA, the prodrug was most effective when the treatment delays were minimized.

Keywords

ADME; Chelation; Oral absorption; Pharmacokinetics; Prodrugs

INTRODUCTION

Americium-241 is a transuranic radionuclide that is found most abundantly in spent nuclear fuel as a byproduct of plutonium processing for the production of nuclear power and nuclear weapons. Widespread exposure to ^{241}Am can occur by its accidental release from nuclear facilities or by the detonation of nuclear devices. It can also be spread on a smaller scale by the illicit detonation of radiological dispersal devices, or “dirty bombs.” Contamination can occur by inhalation, ingestion, or entry through wounds, and internalized ^{241}Am is

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transported in blood before depositing primarily in the skeleton and liver [1–4]. Once deposition occurs, it is difficult to remove the contaminant from the body as current treatments have limited efficacy. The potential for chronic exposure to localized radiation following tissue deposition makes for a hazardous radionuclide that can cause cancer and other radiation-induced injuries.

Intercepting the ^{241}Am in the blood prior to deposition has been proven to be an effective treatment approach. The current first-line treatment is diethylenetriaminepentaacetic acid (DTPA), a chelating agent that is used to facilitate the excretion of internalized radionuclides, including ^{241}Am [5]. The molecular structure of DTPA is provided in Figure 1. The treatment usually involves the intravenous administration of the trisodium calcium or zinc salts of DTPA, after which these chelating agents distribute to the extracellular fluids and are rapidly excreted in urine by glomerular filtration [6,7]. DTPA exhibits a high binding affinity for ^{241}Am with a stability constant of $10^{26.2}$ M^{-1}, enabling it to preferentially bind to the radionuclide in the blood [1]. In the absence of DTPA, plasma proteins and other endogenous ligands in the blood bind to ^{241}Am and facilitate the migration of radionuclide to the deposition sites [3]. DTPA mitigates this risk primarily by capturing the radionuclide prior to tissue deposition and enhancing its urinary excretion.

As an established decorporation agent, DTPA is maintained in the United States Strategic National Stockpile (SNS) in case of radiological and nuclear emergencies. Its efficacy against ^{241}Am contamination has been proven mostly in controlled decorporation studies with animal models [8,9]. Rats and beagles represent the most commonly used animal models, and 30 μmol kg^{-1} has been established as the standard human dose. A single IV bolus dose of DTPA at 30 μmol kg^{-1} in rats administered one hour after IV contamination with ^{241}Am citrate resulted in the cumulative 14-day urinary decorporation of approximately 43% of the injected ^{241}Am, compared to 11% in the control group [10]. An analysis of the ^{241}Am retention in bones, liver, and other tissues confirmed that the DTPA treatment contributed to the significant reduction in tissue accumulation. Similar beneficial effects have been demonstrated with DTPA in beagles following the same weight-normalized dose. The efficacy of DTPA is also evident in humans, most notably in the aftermath of the Hanford americium accident [11]. However, the human subjects were victims of accidental contamination and are not part of controlled studies; thus, the therapeutic effects are not fully characterized.

Despite the supply of DTPA in the SNS, the growing concerns of nuclear terrorism and nuclear accidents in recent years have renewed the interest in the development of new or improved radionuclide decorporation agents [6]. One of the major limitations of DTPA is its need to be administered by intravenous injection or inhalation. Such treatments require skilled medical personnel for administration, and in emergency situations involving mass-casualties, the product is not conducive to rapid distribution and self-administration. DTPA is proven to be most effective when the treatment commences soon after contamination; delaying the treatment can significantly reduce the overall efficacy, which can only be compensated partially by repeated treatments over time [12,13]. An orally administered dosage form can alleviate these limitations. However, the oral absorption of DTPA is hampered by

it hydrophilic nature, resulting in permeability-limited absorption [14]. Thus, the poor oral bioavailability of DTPA makes it unsuitable as a product for oral administration.

To improve the oral delivery of DTPA, a prodrug strategy using the penta-ethyl ester form of DTPA is under investigation. The molecular structure of DTPA penta-ethyl ester is provided in Figure 1. The esterification of the ionizable carboxylic acids of DTPA renders a significantly more lipophilic molecule that can overcome permeability-limited absorption. In contrast to DTPA with a ClogP of −2.7, DTPA penta-ethyl ester has a more favorable ClogP of 4.7 and experimentally measured logD values of 1.5–2.5 under intestinal pH conditions [15]. DTPA penta-ethyl ester is an oily liquid; thus, the bioavailability is not solubility-limited despite its hydrophobicity. Once the prodrug overcomes the permeability barrier of the gut, it is expected that the carboxylic acid esters will undergo chemical and enzymatic hydrolysis to form DTPA. Despite the need for multiple ester cleavages, this complex prodrug is expected to be hydrolyzed extensively, given the prevalence of esterases along the oral absorption pathway [16–18]. Moreover, prior in vivo efficacy studies have already demonstrated the absorption potential of this prodrug, demonstrating enhanced decorporation of ^{241}Am in rats following intravenous and intramuscular contamination [19]. This work further explored the oral absorption potential of this prodrug by examining its pharmacokinetics and biodistribution following the oral administration of DTPA penta-ethyl ester. Additionally, the efficacy of this prodrug was investigated using the inhalation contamination model, an alternative model intended to mimic one of the most likely routes of contamination.

MATERIALS AND METHODS

Test articles

[^{14}C]DTPA penta-ethyl ester was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO) as a stock solution of 37 kBq μL^{-1} in ethanol. The ^{14}C-labels were positioned on the five metabolically stable carbonyl carbon atoms of the DTPA backbone. The molecule possessed a specific activity of 2.04 MBq μmol^{-1}. Non-radiolabeled DTPA penta-ethyl ester was synthesized using the procedure reported previously [20].

^{241}Am nitrate was obtained from the US Department of Energy. An aliquot of the stock solution was dried and reconstituted in 0.25 M nitric acid to produce a contaminating solution of 7.4 kBq mL^{-1} with pH 0.74. The solution was used to create the aerosols for contaminating the rats in the decorporation study.

Protocols for Animal Studies

The pharmacokinetic and biodistribution studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Carolina at Chapel Hill. The decorporation study was approved by the IACUC at Lovelace Respiratory Research Institute, conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* [21].

Pharmacokinetics

The pharmacokinetics of DTPA penta-ethyl ester and its metabolites were evaluated following a single, oral dose of [^{14}C]DTPA penta-ethyl ester in female Sprague Dawley rats (Charles River, Morrisville, NC). Four jugular vein catheterized rats were orally administered by gavage with a DTPA penta-ethyl ester dose of 187 μmol kg^{-1} and 14.8 MBq kg^{-1}. A mixture of radiolabeled and non-radiolabeled DTPA penta-ethyl ester was used to achieve these target doses. The dosing solutions were prepared in a vehicle of pH 4, 25 mM citrate buffer and administered as a solution at a volume of 5 mL kg^{-1}. The rats were fasted for 6 h prior to dosing and housed in individual metabolic cages for the duration of the study. Food was presented 4 h after dosing while water was made freely accessible throughout the study.

The study was carried out for 24 h after administration, at which time the rats were euthanized. Blood and urine were collected at 1, 2, 4, 8, 12, and 24 h during the in-life phase of the study. Blood was obtained via the jugular vein catheters and immediately transferred to collection tubes containing sodium fluoride and potassium oxalate[22]. Upon blood collection, the tubes were inverted several times before centrifuging at 3500 RPM for 5 min to isolate the plasma fraction. For assay, aliquots of plasma and urine were added directly to 10 mL of scintillation cocktail (Ultima Gold, Perkin Elmer, Waltham, MA) and analyzed by liquid scintillation counting (LSC; Tri-Carb 3110 TR, Perkin Elmer). The LSC data were corrected for quenching using an external standard.

The LSC data were used to construct profiles for plasma and urine concentrations of ^{14}C during the study. Since the LSC assay cannot discriminate between the various ethyl esters of DTPA, all ^{14}C-labeled molecules were treated as a single entity for the pharmacokinetic and urinary excretion analyses. As DTPA undergoes minimal metabolism [7], the ^{14}C-labeled molecules were considered to be DTPA or its esterified form. The pharmacokinetic parameters were calculated by non-compartmental analysis using linear trapezoidal calculations (Phoenix WinNonlin, Pharsight, Cary, NC).

In a follow-up pharmacokinetic study, the urine composition was evaluated in an attempt to characterize the extent of in vivo hydrolysis of the prodrug. The study was conducted using 6 rats with the same treatment doses as described previously. Urine samples were collected at 2, 4, 6, 8, 12, and 24 h after oral administration, and the samples were diluted with an equal volume of acetonitrile immediately upon collection and stored on ice until analysis to inhibit post-collection hydrolysis of the ester groups. During preliminary evaluations, this method was determined to stabilize the esters sufficiently to allow for analysis. The samples were analyzed by HPLC (Prominence UFLC, Shimadzu, Columbia, MD) with a flow-through radiochemical detector (Radiomatic 650TR, Perkin Elmer). The HPLC system was equipped with an Alltima C18 (150 × 2.1 mm ID, 5 μm; Grace Discovery Sciences, Deerfield, IL) column operating in a 40 °C oven, and the chromatographic separation was performed at flow rate of 0.25 mL min^{-1} using mobile phases of 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B). The mobile phase composition (A:B) followed a linear gradient from 95:5 to 5:95 over 15 min, followed by an isocratic phase of 5:95 for 5 min. Following the gradient separation, the eluent passed through the in-line radiochemical detector, where it was diluted with 5× volume of flow scintillation

cocktail (Ultima M, Perkin Elmer). Detection and quantification occurred in a 120 μL flow cell using standard measurement parameters for ^{14}C. The separation method was developed during preliminary work using MS detection to identify the various esters.

Biodistribution

A biodistribution study was designed to examine the tissue distribution of ^{14}C following the oral administration of [^{14}C]DPTA penta-ethyl ester. Six female Sprague Dawley rats were monitored in the study using the same treatment conditions as in the pharmacokinetic studies. Urine and feces were collected every 4 h during the in-life phase. Euthanasia was performed 12 h after the administration of the prodrug by overdosing with isofluorane, at which time the whole blood, stomach, small intestines, cecum, colon, mesentery, liver, kidneys were collected and weighed. Preliminary studies confirmed these to be the target organs of accumulation. The urine and plasma (isolated from whole blood) were processed and analyzed in the same manner as described above.

The solid tissues, including feces, were diluted with a 1:1 mixture of acetonitrile and pH 4, 25 mM citrate buffer and homogenized in polycarbonate vials containing steel grinding balls. The homogenization was performed by shaking the vials on the high throughput homogenizer (PowerGen, Thermo Fisher Scientific, Waltham, MA) for 5–8 min at high speeds. Approximately 200 mg of each homogenate was sampled and combined with 1 mL of Solvable (Perkin Elmer) and heated in an oven at 60 °C for 1–3 h until digestion was visibly complete. Once the samples were cooled, hydrogen peroxide (30% in water) was added in two aliquots of 100 μL to de-colorize the sample. The samples were returned to the 60 °C oven for an additional 1 hour. After cooling, each sample was diluted with 10 mL of scintillation cocktail and analyzed by LSC. The radioactivity measured in each sample was weight-corrected to determine the total radioactivity in each whole tissue.

Decorporation Study

The treatment effects of orally administered DTPA penta-ethyl ester were evaluated by examining the decorporation of ^{241}Am in F344 rats (Charles River). The rats were exposed by nose-only inhalation to aerosols of ^{241}Am nitrate. The aerosols were produced with a Hospitec nebulizer and passed through a tube furnace to dry the particles before entering the small animal, nose-only exposure chamber (In-Tox Products, Moriarty, NM). The administered aerosol had a measured ^{241}Am concentration of 34.6 kBq L^{-1} and particle size of 0.61 μm activity median aerodynamic diameter with a geometric standard deviation of 1.66 based on cascade impaction measurements. The animals were exposed to the aerosol for 10 min.

A total of 21 male and 21 female rats were contaminated and divided into seven study groups: four treatment groups and three control groups. The treatment groups were orally administered by gavage with a 374 μmol kg^{-1} dose of DTPA penta-ethyl ester at 1 hour, 1 day, 5 days, or 14 days after ^{241}Am contamination. The DTPA penta-ethyl ester was formulated as a solution in a vehicle of medium-chain triglyceride at a 20% (w/w) concentration. The dosing solution was administered at a volume of 1 mL kg^{-1}. Similarly, the control groups were administered with 1 mL kg^{-1} medium-chain triglyceride vehicle at 1

day, 5 days, or 14 days after ^{241}Am contamination. The rats were housed in metabolic cages for the duration of the study with daily urine and feces collection. Food and water were freely accessible for the duration of the study.

The rats were euthanized at 7 or 8 days post-treatment and necropsy was performed to determine the retention of ^{241}Am in various tissues. The lungs, trachea, tracheobronchial lymph nodes (TBLN), liver, spleen, kidneys, gastrointestinal tract (esophagus, stomach, upper and lower intestines), testes/ovaries, quadriceps, femurs, and soft tissue remains were collected. All tissue samples, except pelt and carcass, were analyzed directly by gamma scintillation counting (2480 Wizard^2 Gamma Counter, Perkin Elmer) for the 59.5 keV photons emitted by ^{241}Am. The pelt and carcass were processed by high temperature ashing (72 h temperature cycles, up to 550 °C) and concentrated nitric acid treatments before gamma scintillation counting. The ^{241}Am retained in total muscle was calculated by measuring the concentration of ^{241}Am in the quadriceps and using it to scale to total muscle. The ^{241}Am concentration in the quadriceps was assumed to be representative of the total muscle, and for the scaling, 0.59 was used as the muscle fraction of the body mass of a rat [24]. The amount of ^{241}Am in the total muscle was subsequently subtracted from the total ^{241}Am recovered in carcass and the amount of ^{241}Am measured in the femurs were added to determine the total ^{241}Am retained in the skeleton. The amount of ^{241}Am in each tissue was calculated as a fraction of the total ^{241}Am recovered from each rat. Analyses for statistical differences to assess treatment effects were performed by one-way ANOVA using JMP 10.0 (SAS Institute, Cary, NC).

RESULTS

Pharmacokinetics

Plasma and urine concentration profiles for ^{14}C were constructed with the LSC data from the pharmacokinetic study. The plasma concentration profile, shown in Figure 2, exhibits an absorption phase that achieves an average maximum plasma level (T_{max}) at approximately 8 h after dosing. Even after 24 h, elevated levels of the ^{14}C remained in the systemic circulation. The systemic exposure ($AUC_{0-\infty}$) following an oral dose of 187 μmol kg^{-1} was calculated to be 974 h nmol mL^{-1} by non-compartmental analysis.

The cumulative urinary excretion profile in Figure 3 shows the excretion of ^{14}C over the 24 h period. The cumulative excretion over this period equates to approximately 33% of the administered dose. Since this quantity does not entirely account for the fraction of the dose that was distributed to tissues or excreted in feces, it can be assumed that the absorbed fraction is likely greater than 33%. Furthermore, from Figure 4, the AUC and urinary excretion within the same sampling intervals show a linear correlation ($R^2 = 0.877$). From the slope of the linear regression, the renal clearance was estimated to be 0.025 L h^{-1}.

A second pharmacokinetic study was conducted to characterize the extent of bioactivation of [^{14}C]DTPA penta-ethyl ester by measuring the urine composition using HPLC with radiochemical detection. The prevalence of esterases along the oral absorption pathway and the absence of metabolism for DTPA suggest that any in vivo modifications to the prodrug should be associated with the hydrolysis of the ester groups. The various DTPA esters that

were measured in the urine are summarized in Figure 5. The chromatographic analysis of the urine revealed the complete metabolism of the penta-ester form as none was detected. Likewise, the tetra-ester form was also not detectable in uine. The analysis revealed that the bioactivation of DTPA penta-ethyl ester was extensive but incomplete. DTPA tri-ethyl ester represented the most abundant intermediate at all time points, and DTPA was the second most abundant. After 24 h, DTPA tri-ethyl ester and DTPA made up 33% and 30% of the total ^{14}C in urine, respectively. These corresponded to 11% and 10% of the administered prodrug dose, respectively. The mono- and di-ester forms collectively accounted for the remaining third of the ^{14}C detected in urine.

Biodistribution

The tissue distribution of ^{14}C was assessed 12 h following the oral administration of [^{14}C]DTPA penta-ethyl ester. The results are summarized in Table 1. Tissue analysis determined that approximately 73% of the administered ^{14}C was associated with the gastrointestinal tract after 12 h. Of this, 29% was found to be associated with the stomach, suggesting that prolonged gastric retention or gastric secretion may have contributed to incomplete absorption. The slow release of the prodrug from the stomach may have contributed to a prolonged duration of absorption, resulting in the sustained plasma profile that was observed in the pharmacokinetic study. In addition to the stomach, high levels of ^{14}C were measured in the caecum and colon, representing approximately 22% and 18% of the administered dose, respectively. Interestingly, despite the high levels in the stomach and colon, the small intestine contained the lowest levels along the gastrointestinal tract, accounting for only 3% of the administered dose. Meanwhile, fecal excretion was negligible.

Of the tissues that do not make up the gastrointestinal tract, the liver exhibited the highest ^{14}C content. However, it contained less than 1% of the administered dose. Approximately 20% of the administered dose was excreted in the urine, consistent with cumulative recovery at 12 h from the pharmacokinetic study. Despite the high excretion of ^{14}C in the urine, the kidney contained negligible amounts. Collectively, a total of 90–110% of the administered ^{14}C was recovered from each rat. This suggests that negligible amounts of DTPA penta-ethyl ester and its hydrolysis products distributed to the remainder of the carcass.

Decorporation

Significant therapeutic effects were observed in rats treated with orally administered DTPA penta-ethyl ester following inhalation exposure to ^{241}Am. The tissue distribution of ^{241}Am following the various treatments is provided in Table 2. The treatment groups recovered 21.7–24.6 kBq of inhaled ^{241}Am from individual rats, and these recovery values were used to determine the fraction of ^{241}Am that were retained in the various tissues. Comparison of the DTPA penta-ethyl ester treatment groups to the corresponding control groups revealed that the prodrug significantly reduced the accumulation of ^{241}Am in certain tissues. This decorporation effect was particularly evident in the liver, lungs, and bone, tissues that are known to be associated with ^{241}Am accumulation. In the case of a 1-hour delay, the improvements corresponded to 11- and 4-fold reduction in the liver and lungs, respectively ($p < 0.01$). At least 2-fold reductions were observed in the bone, gastrointestinal tract,

kidneys, muscle, soft tissues, and spleen ($p < 0.05$). Moreover, the treatment was most effective when the treatment delays were minimized. The apparent high fecal excretion in all treatment groups was attributed to the mucociliary clearance of ^{241}Am as a consequence of the inhalation delivery.

The effects of DTPA penta-ethyl ester in promoting the excretion of ^{241}Am were also clearly evident in the urine data. The daily urinary excretion profiles in Figure 6 show the effects of the prodrug when the treatment was administered 1 h after contamination. The treatment effects were most prominent during the first 3 days after treatment. During this period, the prodrug facilitated the excretion of nearly 18% of the inhaled ^{241}Am, representing nearly 96% of the cumulative excreted total. Over the 8-day duration, a total of 19% of the inhaled ^{241}Am was excreted, corresponding to a nearly 30-fold improvement compared to the control ($p < 0.001$). When treatment delays were extended, the overall decorporation effects were reduced. The effects of treatment delay on decorporation appear in the cumulative excretion profiles of Figure 7. The profiles show that a 2-week delay in treatment reduced the cumulative excretion of ^{241}Am by approximately 5-fold compared to a 1-hour delay ($p < 0.001$). Even so, the treatment facilitated the total decorporation of approximately 4% of the inhaled ^{241}Am, a significant improvement over the control ($p < 0.01$). The prodrug was most effective when the treatment delays were minimized, consistent with observations previously reported for DTPA.

DISCUSSION

In order to overcome the oral delivery challenges of DTPA, the penta-ethyl ester prodrug of DTPA was designed with the intent to increase the lipophilicity of DTPA. Previously reported physicochemical properties of this prodrug revealed that it has significantly higher lipophilicity than DTPA and thus, promising potential for oral delivery [15]. The in vivo studies of this work confirmed this absorption potential, demonstrating that at least 33% of the administered dose was orally absorbed over 24 h based on the urinary excretion data alone. The extent of absorption is significantly higher than orally administered DTPA, which has a reported bioavailability of 3% [14].

The improved oral delivery potential with DTPA penta-ethyl ester is further evident when the pharmacokinetic parameters of the prodrug and DTPA are compared. Following an oral, 187 μmol kg^{-1} dose of the prodrug, DTPA penta-ethyl ester and its hydrolysis products contributed to an $AUC_{0\text{-}\infty}$ and renal clearance of 974 h nmol mL^{-1} and 0.025 L h^{-1}, respectively. In contrast, intravenously administered DTPA at a dose of 29.4 μmol kg^{-1} resulted in an $AUC_{0\text{-}\infty}$ and clearance of 7.1 h nmol mL^{-1} and 0.25 L h^{-1}, respectively [24]. Even with dose-normalization to account for the difference in administered doses, the prodrug exhibited 20-fold higher plasma exposure and a 10-fold lower clearance than DTPA. The significant difference in these pharmacokinetic parameters suggests that the pharmacological activity of the prodrug treatment is likely to be significantly different from DTPA.

While the rate and extent of bioactivation of DTPA penta-ethyl ester remains unknown, the pharmacokinetic behavior following the administration of this prodrug could be favorable

for improving the radionuclide decorporation effects over DTPA. One limitation of DTPA for therapeutic efficacy may be its rapid elimination kinetics in relation to the biokinetics of radionuclides. The primary site of DTPA chelation is in the blood and extracellular fluid (ECF), and while the rapid onset and elimination following a bolus injection of DTPA may be advantageous for facilitating the rapid excretion of contaminants, the chelating agent must be present in the blood and ECF at the appropriate time and at sufficient levels to intercept the contaminant. Thus, it is important for the pharmacokinetics of DTPA to align with the biokinetics of the contaminant. However, not all contamination scenarios manifest themselves in immediate uptake of the contaminant into the systemic circulation. The rate of systemic entry depends on the route of contamination and the properties of the radionuclide, including solubility, charge, and chemical form [25,26]. Incorporation via inhalation and wound are considered to be the two of the most probable routes of radionuclide contamination and are characterized as exhibiting slow or protracted entry of the contaminant into the bloodstream [9,27,28]. Unless frequent injections or continuous infusion of DTPA are administered, its rapid elimination could quickly deplete the available DTPA in the blood and allow for only a limited duration of efficacy. Accordingly, alternative delivery methods with both DTPA and DTPA penta-ethyl ester have been proposed [24,29,30]. An orally bioavailable chelating agent that can provide extended durations of efficacy may be better suited for radionuclide decorporation in a real-life contamination scenario.

Interestingly, the plasma concentration of ^{14}C following the oral administration of [^{14}C]DTPA penta-ethyl ester appears to exhibit a sustained profile. The long residence time of the prodrug and its DTPA intermediates in the plasma is in stark contrast to DTPA, which is known to be eliminated very rapidly with a half life of 0.53 h following an intravenous injection [24]. A possible explanation for the sustained profile is that the prodrug is absorbed over an extended duration contributing to a prolonged influx of ^{14}C into the systemic circulation. Based on the high retention of ^{14}C in the stomach from the biodistribution study, the stomach may be acting as a depot. As the formulation and the administered volume were not unusual for biodistribution studies, these do not appear to be factors in the observed prolonged gastric retention. Previous in vitro evaluations suggested that the prodrug is sufficiently stable against premature chemical hydrolysis in the acidic environment of the stomach[15]. Alternatively, the prodrug may be metabolized in the epithelium, entrapping the DTPA intermediates and slowing its transport to the systemic circulation. Another possibility for the sustained plasma levels may be the result of altered elimination kinetics, a potential consequence of the incomplete conversion of DTPA penta-ethyl ester to DTPA in the plasma. The DTPA intermediates are more lipophilic than DTPA and may have a higher tendency to bind to plasma proteins resulting in lower renal clearance. The intermediates may also distribute more readily to the tissues, further reducing the elimination rate. The combination of longer absorption, larger volumes of distribution and slower elimination may contribute to producing a sustained plasma concentration profile as the one observed with the prodrug treatment.

An analysis of the urine composition confirmed that the bioactivation of DTPA penta-ethyl ester was extensive but incomplete. Preliminary in vitro stability evaluations of DTPA penta-ethyl ester in rat plasma revealed that hydrolysis is very rapid, with complete loss of the penta- and tetra-ester forms occurring within 10 min (data not shown). Thus, it was

considered improbable for any absorbed prodrug to enter the plasma and avoid metabolism before excretion. Unfortunately, an attempt to characterize the composition in plasma was unsuccessful. As part of the pharmacokinetic study, plasma samples were diluted with acetonitrile and analyzed by HPLC using the same methods as described for urine. This sample processing method appeared to sufficiently stabilize the DTPA esters during preliminary in vitro evaluations; however, the data from the in vivo study indicated a nearly complete conversion to DTPA. Since the in vivo study required additional time to isolate the plasma from whole blood before the sample could be stabilized, it is believed that the multi-ester intermediates may have hydrolyzed during this period. Under these circumstances, the composition in the urine was considered to provide the most reliable representation of the extent of bioactivation.

Despite the incomplete bioactivation of DTPA penta-ethyl ester to DTPA, the in vivo decorporation study demonstrated the therapeutic potential of the prodrug. The inhalation route of contamination was chosen in this decorporation study to mimic the route of contamination that may occur in a real-life scenario. Contamination with ^{241}Am nitrate is a realistic hazard and the chemical complex is a moderately soluble form that can slowly release from the lung and into the systemic circulation [9,28,31]. Administering the prodrug 1 h after contamination resulted in an 11-fold decrease in liver accumulation compared to the control treatment in this study. In contrast, as previously reported, administering the same treatment with the same treatment delay after intravenous and intramuscular contamination reduced the accumulation of ^{241}Am in liver by less than 3-fold compared to the corresponding control treatments [19]. Although the extent of actual decorporation may differ due to differences in the amount of accessible contaminant, the relative therapeutic effects in the context of the corresponding controls emphasize the importance of understanding the distribution kinetics of the contaminant to select the appropriate type of treatment.

The observed efficacy in spite of the incomplete hydrolysis of DTPA penta-ethyl ester from urine data suggests that the overall decorporation may be an additive effect produced by the contributions from the various DTPA intermediates. Even with incomplete bioactivation, the DTPA intermediates possess multiple coordination sites to chelate metals in the blood. This potential is exemplified in the established relationship between the number of coordination sites on a chelating agent and the binding of ^{241}Am [32]. The tri-ethyl ester of DTPA, with five coordination sites, represents the DTPA intermediate in the urine with the fewest number of coordination sites. According to this relationship, it may still possess a high affinity for ^{241}Am with a stability constant of approximately 10^{15} M^{-1}. The other intermediates have a greater number of coordination sites and would likely have greater affinities for ^{241}Am. Assuming that this relationship applies for all DTPA intermediates, complete bioactivation of DTPA penta-ethyl ester to DTPA may not be necessary to effectively chelate ^{241}Am in the blood.

In addition to intercepting the radionuclides in the blood, extraction of contaminants from tissues is another means to facilitate decorporation. However, the conventional DTPA treatment is challenged by its limited ability to cross cell membranes and facilitate the excretion of contaminants from the intracellular compartment of tissues [33]. A complete conversion of DTPA penta-ethyl ester to DTPA may not be favorable to facilitate the

removal of deposited radionuclides. Partially hydrolyzed forms of the prodrug are more lipophilic than DTPA and may have the potential advantage to access the intracellular space, thereby enhancing the extraction of radionuclides from the deposition sites. The penta-ethyl ester of DTPA was previously administered by intraperitoneal injection to explore this decorporation mechanism and was proven to facilitate intracellular decorporation of ^{239}Pu [33]. This type of mechanism may be particularly advantageous when the treatment following contamination is delayed and extensive tissue deposition has already occurred. This effect appears to be evident in the decorporation study reported here which showed that the ^{241}Am retention in the primary deposition sites of lung, liver, and bone were reduced significantly even after a 14-day delay in the treatment with the prodrug. With an intracellular decorporation mechanism, repeated administration of this prodrug may further add to the therapeutic effects by extending the ability to extract the radioactive contaminants from the deposition sites.

The DTPA penta-ethyl ester treatments were most efficacious when the treatment delays after contamination were minimized. An extension of the treatment delay from 1 h to 1 day resulted a 2-fold reduction in ^{241}Am decorporation. The decrease in the therapeutic effect became less apparent with longer treatment delays. This overall trend is consistent with the effects observed after conventional DTPA treatments, which relies primarily on intercepting the radionuclides before tissue deposition. Even though the incomplete bioactivation of the prodrug may enhance the intracellular decorporation of radionuclides, capturing the contaminants before deposition appears to be the most effective mechanism for decorporation. The diminished effects after longer treatment delays with this prodrug reflect the need to still provide urgent treatment to victims of contamination.

CONCLUSIONS

The pharmacokinetics, biodistribution and efficacy of orally administered DTPA penta-ethyl ester were investigated in rats. A single dose treatment in the pharmacokinetics study revealed that greater than 33% of the administered dose was absorbed based on urinary excretion data alone. From the urine composition, the bioactivation was determined to be extensive but incomplete. The plasma concentration profile showed elevated plasma levels of the prodrug and its hydrolysis products for 24 h. The biodistribution study showed that the prodrug and the intermediates were primarily retained in the gastrointestinal tract with limited distribution to other tissues after 12 h. In the efficacy study, a single dose treatment of DTPA penta-ethyl ester administered after contamination enhanced the decorporation of inhaled ^{241}Am by as much as 15-fold compared to the control treatment. Consistent with prior reports of DTPA, the therapeutic effects of the prodrug were maximized when the treatment delays were minimized. Overall, DTPA penta-ethyl ester exhibited promising pharmacokinetic and therapeutic behavior as an orally administered radionuclide decorporation agent.

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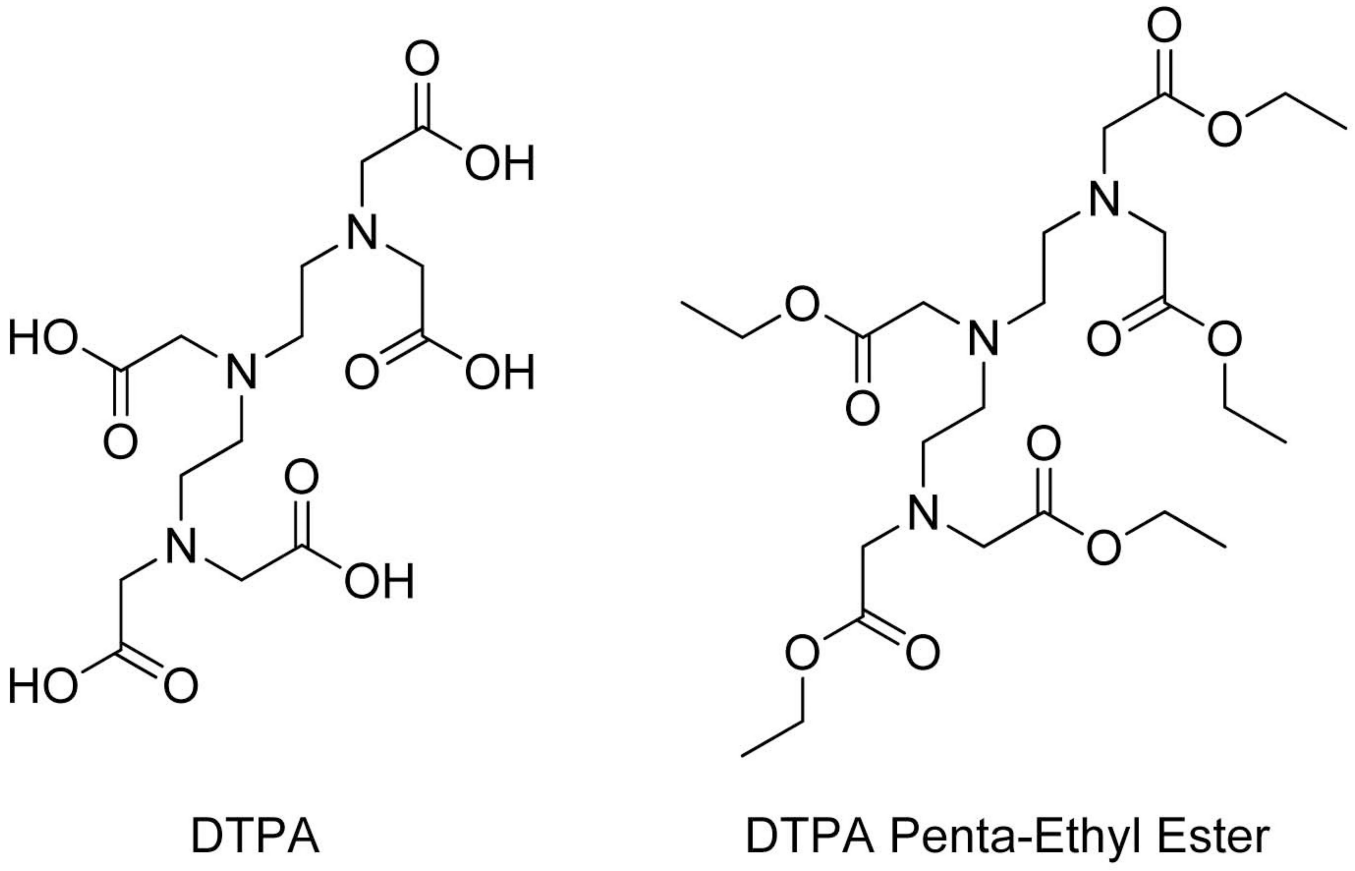

Figure 1.
Structures of DTPA and DTPA penta-ethyl ester

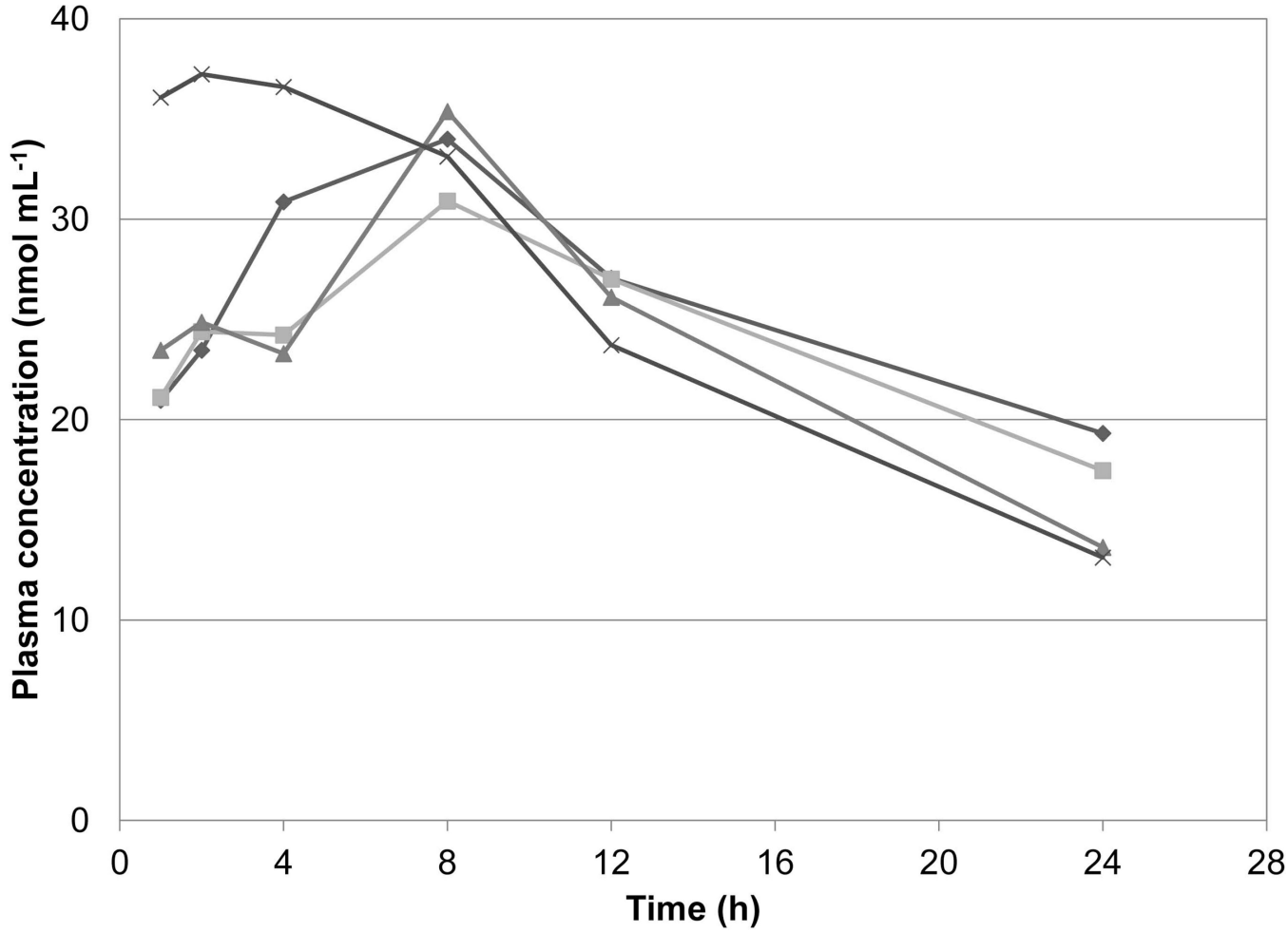

Figure 2.

Plasma concentration of ^{14}C in individual rats from the oral administration of [^{14}C]DTPA penta-ethyl ester

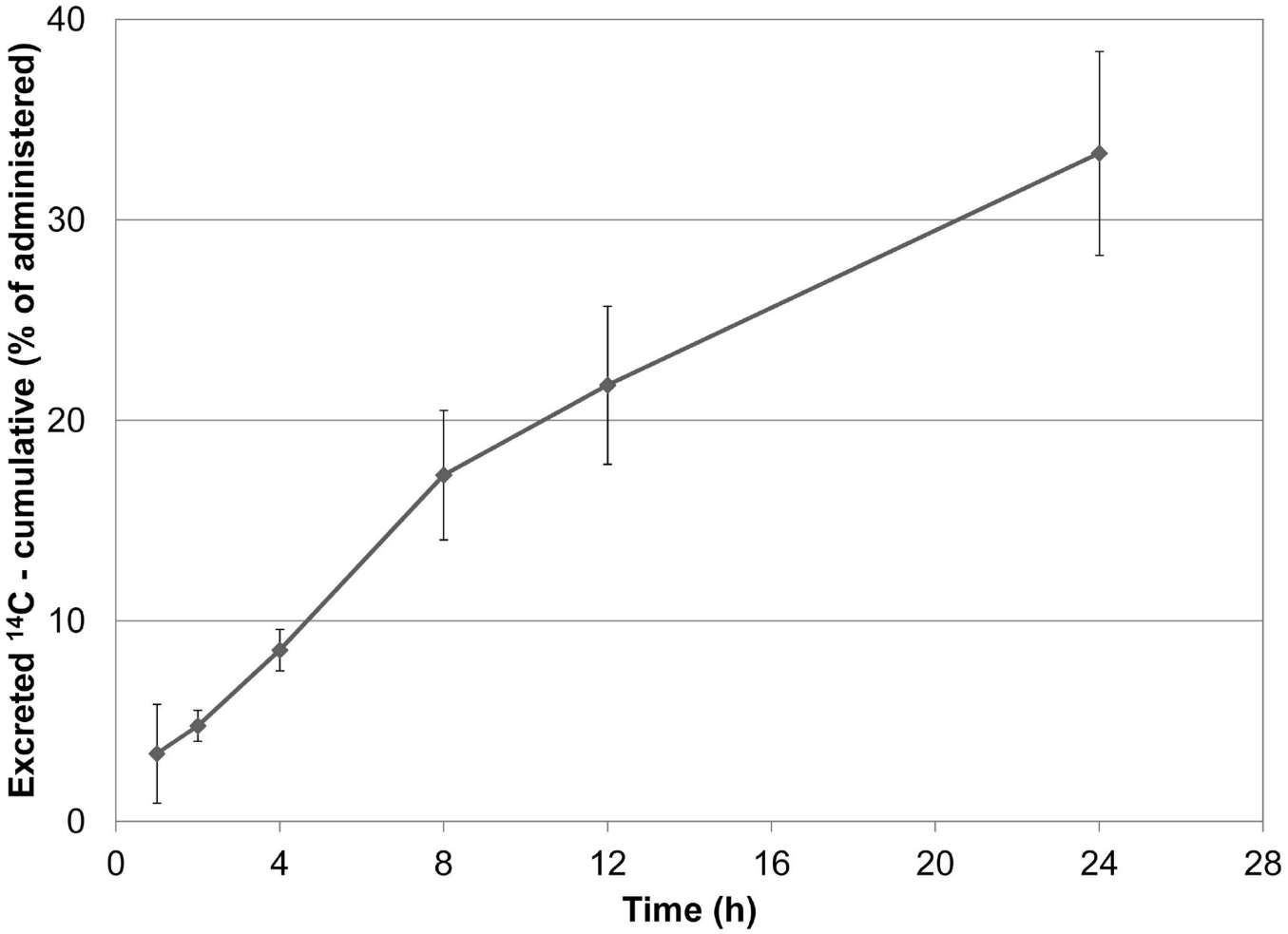

Figure 3.

Cumulative urinary excretion of ^{14}C from the oral administration of [^{14}C]DTPA penta-ethyl ester (error bars represent 95% CI about the mean)

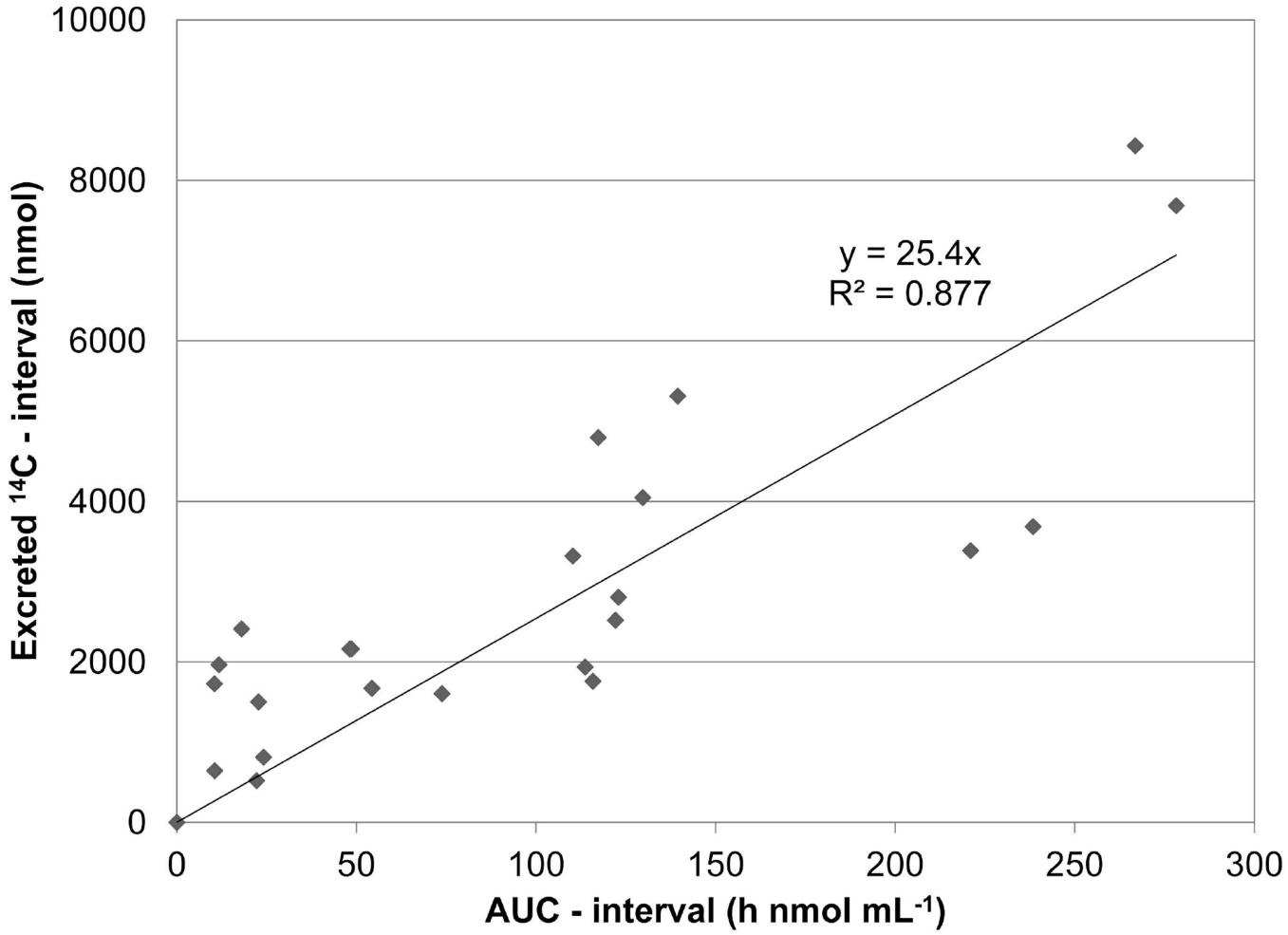

Figure 4.

Correlation between AUC within plasma sampling intervals and urinary excretion within urine collection intervals following the oral administration of [^{14}C]DTPA penta-ethyl ester

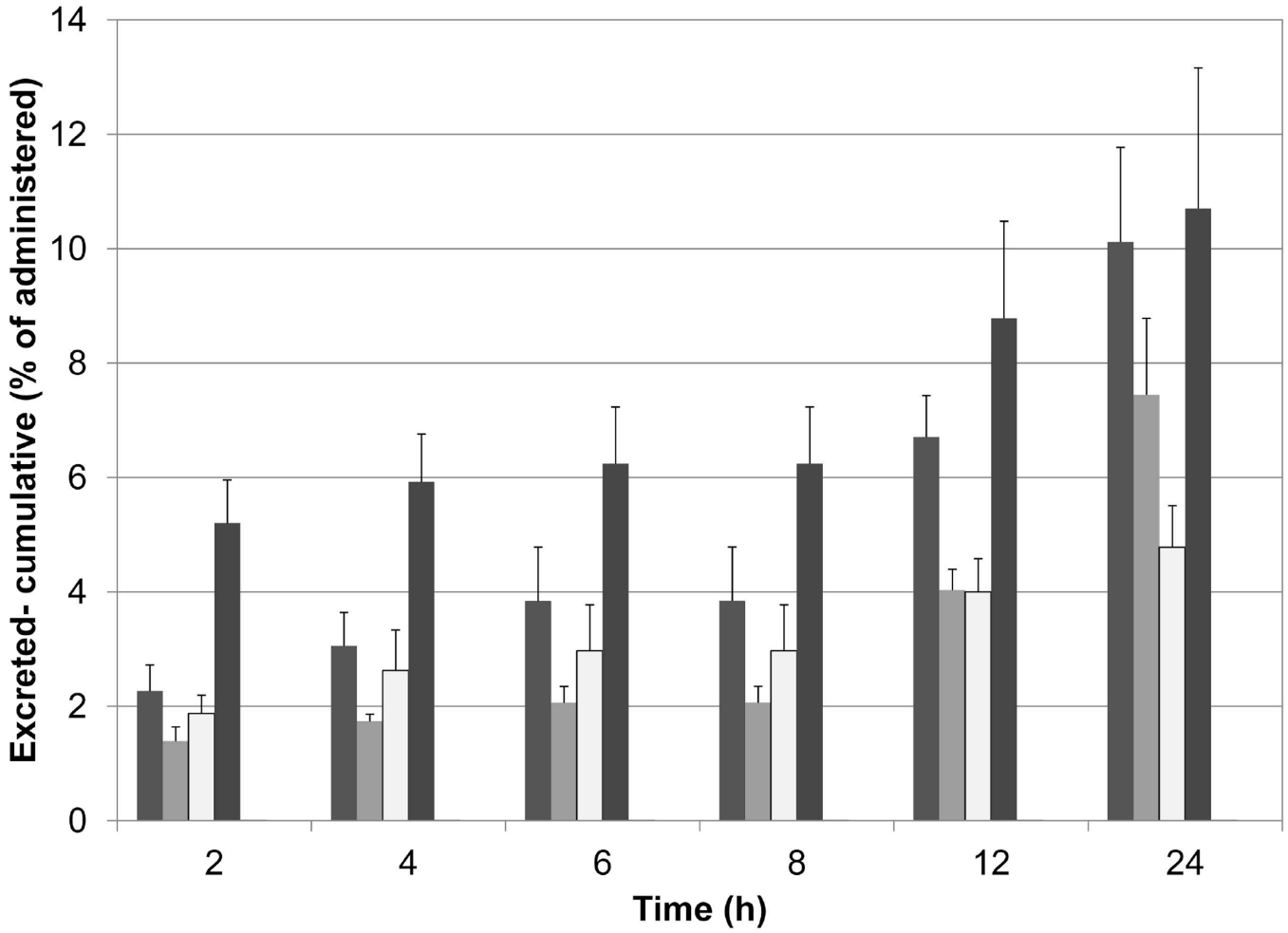

Figure 5.

Composition of the DTPA penta-ethyl ester bioactivation products in urine (error bars represent 95% CI about the mean): DTPA (■); DTPA mono-ethyl ester (■); DTPA di-ethyl ester (□); DTPA tri-ethyl ester (■); DTPA tetra-ethyl ester and DTPA penta-ethyl ester (not detected)

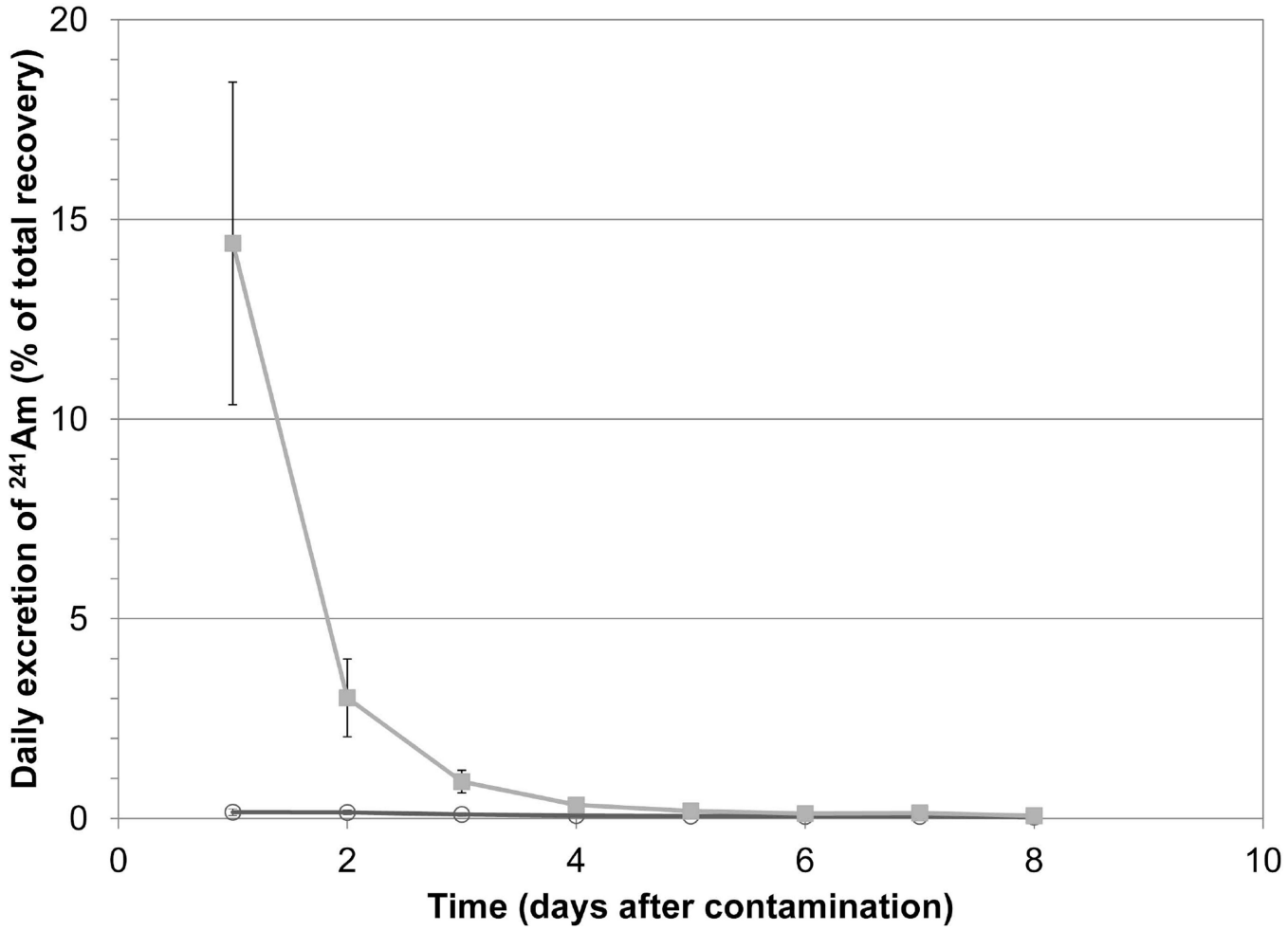

Figure 6.

Daily urinary excretion of ^{241}Am (error bars represent 95% CI about the mean): 1-hour treatment delay with prodrug (—■—) and control (—○—)

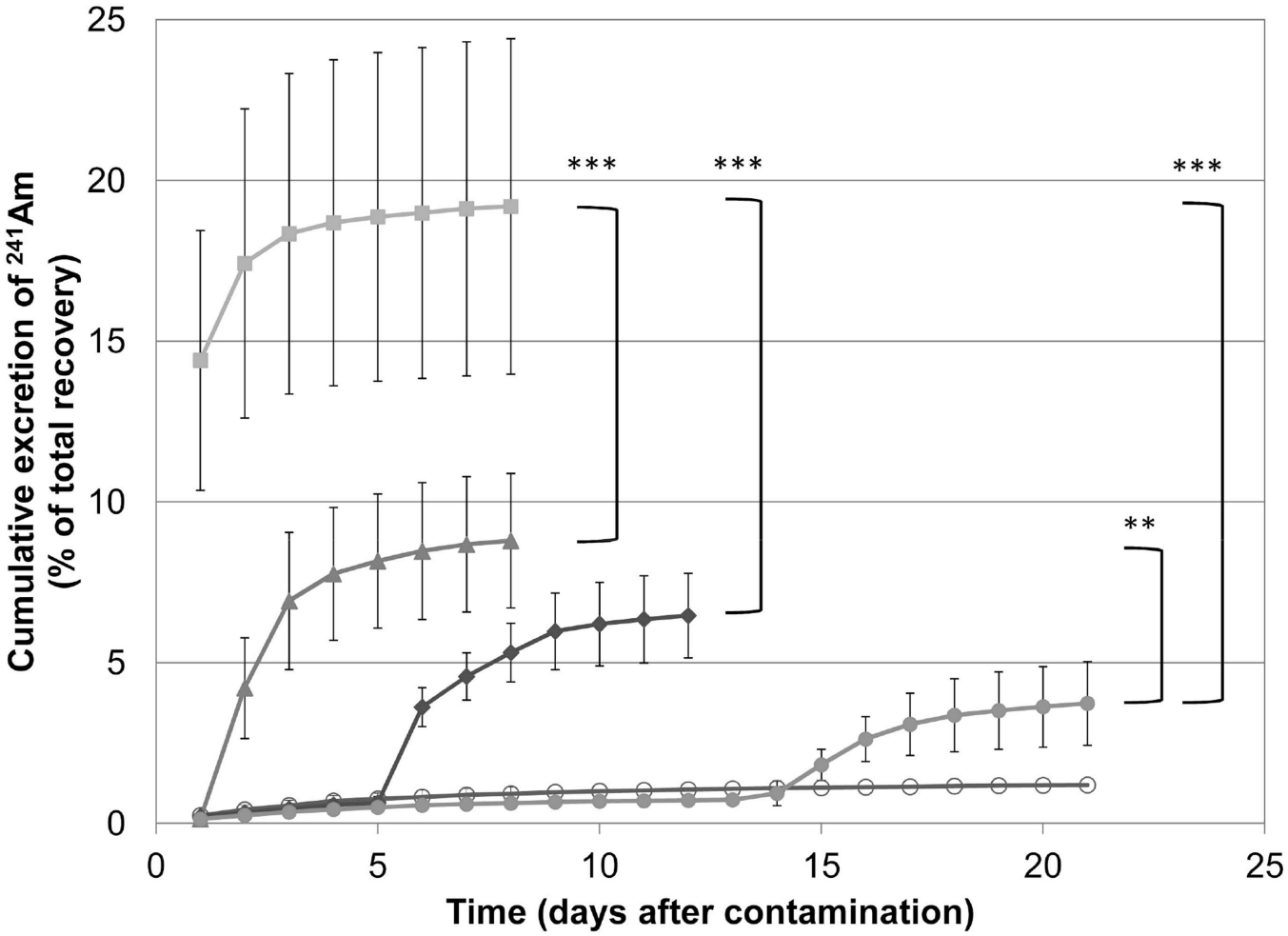

Figure 7.

Cumulative urinary excretion of ^{241}Am by treatment group (error bars represent 95% CI about the mean): 1-hour treatment delay (); 1-day treatment delay (); 5-day treatment delay (); 14-day treatment delay (); control (). Statistical difference between treatment groups determined on the cumulative total: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Tables 1

Tissue distribution of ^{14}C following the oral administration of [^{14}C]DTPA penta-ethyl ester

Tissue	Recovery (%, mean ± 95% CI)
Caecum	22.2 ± 8.7
Colon	18.5 ± 7.1
Feces	0.68 ± 1.13
Kidneys	0.147 ± 0.057
Liver	0.759 ± 0.152
Mesentery	0.039 ± 0.008
Small intestines	3.33 ± 0.94
Spleen	0.010 ± 0.007
Stomach	28.9 ± 10.5
Urine	20.5 ± 5.1

Table 2

Tissue distribution of inhaled ^{241}Am following treatment with orally administered DTPA penta-ethyl ester with varying treatment delays

Tissue	^{241}Am Recovery by Treatment Type (%, mean ± 95% CI)						
	Control, 1 d delay	Prodrug, 1 h delay	Prodrug, 1 d delay	Control, 5 d delay	Prodrug, 5 d delay	Control, 14 d delay	Prodrug, 14 d delay
Blood	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Bone	4.80 ± 2.01	2.10** ± 0.16	2.86* ± 0.68	5.72 ± 1.57	3.92* ± 0.50	6.18 ± 1.56	5.19 ± 1.29
Feces	71.6 ± 10.7	72.6 ± 5.3	77.4 ± 2.4	73.5 ± 5.5	78.2 ± 3.62	79.1 ± 4.6	81.7 ± 3.1
GI tract	1.17 ± 0.42	0.450** ± 0.086	0.832 ± 0.215	1.12 ± 0.21	0.636*** ± 0.109	0.741 ± 0.184	0.575 ± 0.115
Kidneys	0.607 ± 0.231	0.219** ± 0.065	0.353* ± 0.20	0.708 ± 0.249	0.523 ± 0.152	0.650 ± 0.243	0.547 ± 0.144
Liver	7.65 ± 3.95	0.693** ± 0.253	1.59** ± 1.14	7.32 ± 2.69	2.94** ± 1.30	5.37 ± 1.78	3.21 ± 2.06
Lungs	10.1 ± 3.5	2.47** ± 0.36	4.55** ± 1.29	8.44 ± 1.24	4.63*** ± 0.89	5.34 ± 1.32	3.75* ± 0.59
Muscle	0.469 ± 0.332	0.165* ± 0.053	0.332 ± 0.064	0.561 ± 0.111	0.362** ± 0.102	0.460 ± 0.130	0.416 ± 0.059
Ovaries	0.006 ± 0.005	0.002 ± 0.002	0.004 ± 0.001	0.006 ± 0.004	0.003 ± 0.003	0.005 ± 0.004	0.005 ± 0.002
Pelt	2.71 ± 1.57	2.02 ± 0.70	3.15 ± 1.32	1.47 ± 0.40	1.30 ± 0.56	0.748 ± 0.105	0.660 ± 0.170
Soft tissue	0.160 ± 0.053	0.052** ± 0.016	0.093* ± 0.025	0.167 ± 0.096	0.151 ± 0.082	0.171 ± 0.051	0.138 ± 0.051
Spleen	0.020 ± 0.010	0.007** ± 0.001	0.014 ± 0.005	0.026 ± 0.006	0.025 ± 0.011	0.029 ± 0.008	0.039 ± 0.013
TBLN	0.001 ± 0.004	0.001 ± 0.001	0.001 ± 0.001	0.004 ± 0.002	0.002 ± 0.002	0.001 ± 0.001	0.001 ± 0.001
Testes	0.019 ± 0.017	0.008 ± 0.007	0.011 ± 0.004	0.025 ± 0.008	0.020 ± 0.004	0.027 ± 0.029	0.022 ± 0.016
Urine	0.651 ± 0.194	19.2*** ± 5.2	8.80*** ± 2.09	0.942 ± 0.324	7.34*** ± 2.49	1.19 ± 0.12	3.73*** ± 1.30

Statistical significance of the prodrug treatment compared to control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

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