Research Article

Radionuclide Decorporation: Matching the Biokinetics of Actinides by Transdermal Delivery of Pro-chelators

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Abstract. The threat of nuclear terrorism by the deliberate detonation of a nuclear weapon or radiological dispersion device (“dirty bomb”) has made emergency response planning a priority. The only FDA-approved treatments for contamination with isotopes of the transuranic elements Am, Pu, and Cm are the Ca and Zn salts of diethylenetriaminepentaacetic acid (DTPA). These injectable products are not well suited for use in a mass contamination scenario as they require skilled professionals for their administration and are rapidly cleared from the circulation. To overcome the mismatch in the pharmacokinetics of the DTPA and the biokinetics of these transuranic elements, which are slowly released from contamination sites, the penta-ethyl ester of DTPA (C2E5) was prepared and formulated in a nonaqueous gel for transdermal administration. When gels comprised of 40% C2E5, 40–45% Miglyol® 840, and 15–20% ethyl cellulose were spiked with [¹⁴C]-C2E5 and applied to rat skin; over 60% of the applied dose was absorbed within a 24-h period. Radioactivity was observed in urinary and fecal excretions for over 3 days after removal of the gel. Using an ²⁴¹Am wound contamination model, transdermal C2E5 gels were able to enhance total body elimination and reduce the liver and skeletal burden of ²⁴¹Am in a dose-dependent manner. The efficacy achieved by a single 1,000 mg/kg dose to contaminated rats was statistically comparable to intravenous Ca-DTPA at 14 mg/kg. The effectiveness of this treatment, favorable sustained release profile of pro-chelators, and ease of administration support its use following radiological emergencies and for its inclusion in the Strategic National Stockpile.

KEY WORDS: chelating agent; decorporation; DTPA; gel; transdermal drug delivery.

INTRODUCTION

The increasing threat of nuclear terrorism as well as incidents that involved the release of radioactive materials into the environment, such as the accident at the Fukushima Daiichi power plant in March 2011, has heightened the awareness for many nations for the need to be prepared for such cataclysmic events (1). Detonation of a nuclear weapon or a radiological dispersion device (“dirty bomb”) near densely populated areas could result in a large number of individuals being contaminated by radionuclides *via* inhalation, ingestion, or through wounds. Internalization of radioactive materials may result in acute radiation sickness or chronic injuries including an increased risk of developing cancers (2–5). Due to their abundance and availability, isotopes of the transuranic elements americium (Am), curium (Cm), and plutonium (Pu) are among the radionuclides of greatest concern with respect to accidental or deliberate contamination. The injuries and risks associated with

contamination by these radionuclides can be mitigated by the intravenous (i.v.) administration of radionuclide decorporation agents such as the calcium (Ca) and zinc (Zn) trisodium salts of diethylenetriaminepentaacetic acid (DTPA). Ca-DTPA and Zn-DTPA exert their pharmacological effect by sequestering these radionuclides with high affinity and promoting the elimination of the resulting chelate complexes from contaminated individuals. DTPA is administered *via* slow i.v. push, i.v. infusion, or inhalation with a nebulizer (6); treatment is most effective when administered shortly after contamination before the transuranic radionuclides become fixed in tissues such as liver and bone (7). The efficacy of Ca/Zn-DTPA injections has been demonstrated for decades in workers injured in accidents in the nuclear power industry (3,8–10).

Mass contamination scenarios call for effective and prompt medical countermeasures for the affected populations. Current DTPA treatment options do not meet the challenge imposed by a mass casualty setting, in that skilled medical professionals who may not be available are required to administer Ca/Zn-DTPA by i.v. injection. Moreover, multiple injections may be required due to the short circulating half-life of DTPA. Stevens and colleagues studied the clearance of DTPA in man and observed that intravenously administered ¹⁴C-labeled DTPA was quantitatively excreted intact in urine in 24 h (11). The total body clearance of

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^{14}C -labeled DTPA in rats 24 h after i.v. injection has been reported to range from 94 to 100% with half-lives from 0.28 to 0.53 h and with no metabolic degradation (12,13). In contrast, the release rates of internalized Am, Pu, and Cm contaminants from wound sites to the systemic circulation in various animal species range from 0.052 to 6.3% of the administered radionuclides per day, a relatively slow and steady transfer process (14).

It has been suggested that a chelating agent must be maintained at a concentration of at least 10 to 25 μM in both extracellular and intercellular fluids for a sustained duration to ensure an optimal chelation effect of transuranic radionuclides (15). In comparing the short half-life and rapid clearance of DTPA after i.v. administration to the slow and sustained introduction of radioactinides into the systemic circulation (14), there is a mismatch between the pharmacokinetic profile of intravenously administered DTPA and the biokinetic profile of transuranic radionuclides. This mismatch leads to a period where DTPA plasma concentrations are below the effective concentration required to chelate radionuclides in the systemic circulation and, thus, may limit the effectiveness of the current parenteral DTPA treatments. Previous efforts have addressed this mismatch and produced encouraging results. Guilmette and Muggenburg implanted subcutaneous osmotic pumps to continuously deliver Zn-DTPA to dogs that had been contaminated with $^{241}\text{AmO}_2$ by inhalation, and achieved enhanced decorporation of ^{241}Am (16). DTPA has been entrapped in various liposome formulations for prolonged circulation after i.v. administration, and improved decorporation of ^{238}Pu was achieved (13,17). However, these approaches involve parenteral administration, thus making them unsuitable for mass casualty scenarios after a major nuclear/radiological emergency. As a result, a nonparenteral delivery system which can provide sustained levels of chelators in the circulation that match the biokinetic profile of actinides after inhalation or wound contamination is highly desirable.

There are several efforts underway to improve the oral bioavailability of Ca/Zn DTPA and other actinide decorporation agents (18). DTPA is a synthetic polyamino carboxylic acid with eight coordinate bond forming sites for complexing metal ions. Due to the presence of five carboxylic acid groups and three nitrogen atoms, DTPA is highly ionic. Thus, Ca-DTPA and Zn-DTPA are hydrophilic and are considered Class III compounds (high solubility, low permeability) according to the Biopharmaceutical Classification System. While oral formulations may provide for sustained blood concentrations of DTPA, a zero-order release profile can be achieved *via* transdermal drug delivery. Because DTPA is hydrophilic ($\log P_{\text{oct/water}} = -4.90$) with a high melting point (219–220°C), it is not a good candidate for transdermal delivery (19,20). However, esterification of the five carboxylic groups on DTPA produces lipophilic compounds which possess desirable physicochemical properties for transdermal delivery (21,22). We have prepared the penta-ethyl ester of DTPA, designated as C2E5, and successfully incorporated it into a nonaqueous gel formulation comprised of ethyl cellulose (EC) and Miglyol® 840 with acceptable stability and rheological properties (23). The selected 40% C2E5 nonaqueous gel was applied to Sprague–Dawley (SD) rats at a dose of 200 mg/kg C2E5

and achieved sustained release of C2E5 metabolites *in vivo* for an extended duration (23).

The aim of the current studies was to assess the transdermal absorption and excretion of C2E5 nonaqueous gels and evaluate the radionuclide decorporation efficacy of transdermal C2E5 nonaqueous gels at different dose levels when applied 24 h after contamination using an ^{241}Am wound contamination model. To our knowledge, this is the first report of the use of a transdermal formulation for the systemic delivery of a radionuclide decorporation agent with the goal of providing a sustained release pharmacokinetic profile of a chelator in order to match the biokinetic profile of internalized actinides.

MATERIALS AND METHODS

Material

Miglyol 840 was purchased from Sasol (Hamburg, Germany). EC (Ethocel Std 10 FP Premium polymer) with an ethoxyl content of 48.0–49.5% was a gift from Dow Chemical (Midland, MI, USA). C2E5 was prepared using the Fischer esterification method by reacting DTPA with ethanol under reflux in the presence of a hydrochloric acid catalyst (22). [^{14}C]-diethylenetriaminepentaacetic acid penta-ethyl ester ([^{14}C]-C2E5; 50 mCi/mmol) labeled at carbon-1 (carbonyl carbon) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). [^{241}Am]-americium nitrate solution for intramuscular (i.m.) contamination of adult female SD rats was prepared from [^{241}Am]-americium chloride (Eckert & Ziegler Isotope Products, Valencia, CA) by dilution with a solution of concentrated nitric acid. Anhydrous ethanol, isopropyl alcohol, and 30% hydrogen peroxide solution were purchased from VWR International (Radnor, PA) and/or Fisher Scientific (Fairlawn, NJ). Liquid scintillation cocktails Ultima Gold^{TM} and an aqueous-based tissue solubilizer Solvable^{TM} were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA).

Preparation of C2E5 Nonaqueous Gel

C2E5 nonaqueous gels comprised of 40% C2E5, 15–20% EC, and 40–45% Miglyol 840 were prepared using the solvent evaporation method described previously (23). Briefly, pre-dried EC particles were first dissolved in anhydrous ethanol (10% w/v of EC in ethanol) to form a clear solution. This was followed by addition of Miglyol 840 and C2E5 to form a homogenous solution under stirring and subsequently removing ethanol under vacuum to yield the C2E5 nonaqueous gel.

[^{14}C]-labeled C2E5 nonaqueous gel was prepared by adding [^{14}C]-C2E5 (50 mCi/mmol) into the C2E5 nonaqueous gels followed by mixing. The [^{14}C] content of the nonaqueous gel was quantified by adding a fixed quantity of the gel with 10 mL of Ultima Gold^{TM} scintillation cocktail and counting directly for radioactivity by liquid scintillation counting (LSC) using a Packard TriCarb 3100TR (PerkinElmer Life and Analytical Sciences), with automatic quench correction. Samples were counted for 10 min or until a 5% confidence level was achieved. The specific activity of [^{14}C]-labeled C2E5 nonaqueous gel was calculated by dividing sample radioactivity by total sample mass.

In Vivo Studies

Animals

All animal studies were conducted according to a protocol approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee. Ten-week-old adult female SD rats weighing from 200 to 300 g were used in these studies (Charles River Labs, Raleigh, NC). Food and water were given *ad libitum*. The animal room was kept at a controlled temperature on a 12/12 h light/dark cycle (light exposure from 7 a.m. to 7 p.m.). For the duration of the study, the rats were housed in metabolic cages individually with daily urine and feces collection until euthanasia on day 6 for mass balance study and on day 7 for radionuclide decorporation study.

Absorption and Mass Balance Study with [^{14}C]-Labeled C2E5 Nonaqueous Gels

To evaluate the absorption and mass balance of transdermal delivery of the C2E5 nonaqueous gel, the [^{14}C]-labeled C2E5 nonaqueous gel was applied to rats at a 200 mg/kg C2E5 dose level. Six adult female SD rats were anesthetized with 2–3% isoflurane before the dorsal skin between the cervical vertebrae, and anterior thoracic vertebrae was clipped with caution to avoid irritation of the skin that could increase absorption properties. The [^{14}C]-labeled gel was applied to a 2 cm $\times$ 3 cm region using a cotton swab. The mass of the gel applied was recorded for each rat to permit actual dose determination, and a jacket with a dermal insert (VWR International, Radnor, PA) was placed on the rats to protect the area on which the gel was applied. Twenty-four hours after application, the remaining gel at the application site and the jacket with the dermal inserts were carefully removed, and the recovered C2E5 content was assayed by LSC. The animals were housed in metabolic cages individually and were euthanized 6 days after application of the gel. The animals were observed once daily and their body weights recorded at pre-dose and prior to necropsy. Urine and feces were collected daily until euthanasia on day 6, when the liver and the skin from the application site and surrounding area were also collected. Cage washes were collected at the end of each experiment.

Collected urine samples were added to Ultima Gold^{TM} scintillation cocktail at a ratio of 100 μL :10 mL and assayed directly for radioactivity by LSC. For feces samples, the fecal pellets were mixed and vortexed with 20 mL of a mixture of acetonitrile and water (1:1 ratio) for 10 min, followed by a 30 min sonication, and subsequently centrifugation at 1,000 $\times$ g at 4°C for 10 min. The supernatant was transferred to a scintillation vial for analysis by LSC (100 μL of feces extraction supernatant sample directly dispersed into 10 mL of scintillation cocktail). The extraction process was repeated until the sample count was less than 1,000 disintegrations per minute per 100 μL of feces extraction supernatant sample. The liquid cage wash samples were processed in a manner similar to the urine samples and assayed by LSC; the solid cage wastes were processed as fecal pellets and assayed by LSC. For skin and liver samples, tissues were mixed with tissue solubilizer (10% w/v of tissue to solvable) and incubated at 50°C until the tissues became totally

solubilized. One milliliter of the solubilized tissue solution was transferred to a vial, decolorized by incubation with 0.1 mL of 30% hydrogen peroxide solution for 1 h at 50°C, and then added to 10 mL of scintillation cocktail for LSC analysis.

Radionuclide Decorporation of Contaminated Rats

To evaluate the efficacy of transdermal delivery of C2E5 in a nonaqueous gel, a radionuclide decorporation efficacy study was conducted in rats contaminated with ^{241}Am . Adult female SD rats were anesthetized with 2–3% isoflurane. All animals were contaminated with [^{241}Am]-americium nitrate solution (250 mCi, 0.1 mL) *via* an i.m. injection in the anterior thigh muscle. Dorsal skin between the cervical vertebrae and anterior thoracic vertebrae was clipped with caution before application of the gel. C2E5 doses of 200, 600, and 1,000 mg/kg were applied 24 h post ^{241}Am contamination to a 6 cm ^{2} (2 cm $\times$ 3 cm) area of the clipped dorsal region using cotton swab. Fick's law (Eq. 1) dictates the steady-state diffusion of drug through skin,

$$J_{ss} = \left(\frac{D * K_p}{h} \right) * A * C_{veh} \quad (1)$$

Where J_{ss} =steady-state flux (in milligrams per hour), D =drug diffusivity (in square centimeters per hour), h =membrane thickness (in centimeters), K_p =drug's membrane-vehicle partition coefficient, C_{veh} =initial drug concentration (in milligrams per cubic centimeter) in the vehicle, and A =surface area (in square centimeters). Keeping all other parameters constant, the flux of the drug (J_{ss}) is proportional to the surface area of drug application on the skin. To assess the effect of application area, the C2E5 nonaqueous gel was applied at a dose of 600 mg/kg dose 24 h post ^{241}Am contamination using cotton swab to an 18 cm ^{2} (3 cm $\times$ 6 cm) area of the clipped dorsal region. A jacket with plastic dorsal insert was placed on the rats to protect the gel application region. The mass of C2E5 gel applied was recorded for each animal to permit the actual dose determination. Twenty-four hours after application of the C2E5 gel, the remaining gel at the application site and the jacket with the dermal insert were carefully removed. The animals were housed in metabolic cages individually and were euthanized 7 days after contamination. Positive and negative controls included animals administered with Ca-DTPA intravenously at a dose of 14 mg/kg 24 h after contamination and animals untreated with any decorporation agent. Daily observations and body weights were recorded at pre-dose and prior to necropsy. Urine and feces were collected daily until euthanasia, then the liver, kidneys, both femurs, the muscle tissue around both femurs, and the pelt around the ^{241}Am injection site were also collected. Cage washes were collected at the end of each experiment. As ~35% of the decay of ^{241}Am is associated with photon emissions of 59.7 keV, ^{241}Am present in samples was quantified using a gamma counter (2470 Wizard 2, Perkin Elmer). The samples were counted for 1 min using a 40–80 keV energy detection window and were background-corrected. Additionally, ^{241}Am activity was quantified in 2 $\times$ 0.1 mL aliquots of the dosing solution. For all samples, ^{241}Am content was expressed as a percentage of the injected dose. An estimate of the total skeletal burden was made using the method of Volf that was determined by the ^{241}Am burden in the contralateral femur multiplied by 20 (24). The ^{241}Am retained at the wound site was quantified by measuring the ^{241}Am content of the muscle surrounding the injection site plus the ^{241}Am

content in the femur. An estimate of muscle burden was calculated based on the assumption that it represents 45% of the body weight (24). The ^{241}Am content of the muscle from the opposite hind leg and animal body weight at sacrifice were used to determine the estimated ^{241}Am burden in the muscle. Total recovery = total decorporation + liver ^{241}Am content + kidney ^{241}Am content + (skeleton ^{241}Am content - ^{241}Am in contralateral femur) + ^{241}Am content at the wound site + muscle ^{241}Am content + ^{241}Am content at the pelt around the ^{241}Am injection site.

Statistical Analysis

All data reported are mean $\pm$ standard deviation from a given number of observations. Statistical analysis was performed on the data from the efficacy study. To assess the treatment and dose-induced changes in endpoint ^{241}Am data, *i.e.*, liver, skeleton, and wound site burden as well as total decorporation, one-way ANOVA with Tukey's posttest was performed among three C2E5 transdermal treatment groups at 200, 600, and 1,000 mg/kg doses applied to a 6 cm² application site, an untreated negative control group, and a positive control group that received *i.v.* Ca-DTPA. A second one-way ANOVA with Tukey's posttest was performed among all the four C2E5 gel treatment groups, negative untreated, and positive *i.v.* DTPA treatment groups. To assess the sustained efficacy of transdermal C2E5 treatment, daily ^{241}Am decorporation in the urine and feces was analyzed by repeated measures one-way ANOVA with Dunnett's posttest to compare each C2E5 treatment group with the untreated control group. For all statistical analysis, $p < 0.05$ was considered significant. Statistical analyses were performed using the SAS analysis system (v. 9.3; SAS Institute, Inc., Cary, NC).

RESULTS

Preparation of C2E5 Nonaqueous Gels

The structures of C2E5, its potential metabolites, including DTPA tetra-ethyl ester (C2E4), DTPA triethyl ester (C2E3), DTPA diethyl ester (C2E2), DTPA monoethyl ester (C2E1), and the fully de-esterified metabolite (DTPA) are depicted in Fig. 1. C2E5 is a clear, light yellow, slightly viscous Newtonian liquid with a viscosity of ~ 175 cP (22).

C2E5 nonaqueous gels comprised of 40% C2E5, 15–20% EC, and 40–45% Miglyol 840 were prepared using the solvent evaporation method described previously (23). The C2E5 gels were slightly yellow translucent semisolids with a density of 1.02 g/cm³. C2E5 nonaqueous gels comprised of 40% C2E5, 15–20% EC, and 40–45% Miglyol 840 were radiolabeled with [^{14}C]-C2E5 (specific activity = 0.10 $\mu\text{Ci}/\text{mg}$) for a mass balance study. All gels were stored at 4°C until applied to experimental animals.

Absorption and Mass Balance of [^{14}C]-Labeled C2E5 Nonaqueous Gels

Significant C2E5 transdermal absorption was achieved after topical application of the gels; approximately 32% of the 200 mg/kg C2E5 applied dose was recovered from the skin as unabsorbed dose 24 h after application (Table I). Six days after application, a [^{14}C]-C2E5 mass balance of over 90% was obtained, with just

under 90% of the absorbed dose recovered in the excreta and tissues (Table I). Low retention of C2E5 and its metabolites was observed at the application site; only 1.6% of the applied dose was present in the skin taken from the application site 6 days after application (Table I). There was no significant [^{14}C] radioactivity remaining in the liver 6 days after application of the radiolabeled gel, suggesting that C2E5 and its metabolites had relatively short half-lives in plasma.

As demonstrated in Fig. 2, the urinary elimination of [^{14}C] radioactivity peaked 1 to 2 days after application and gradually declined to the baseline level by the sixth day; the fecal elimination of [^{14}C] radioactivity peaked 2 days after administration and gradually declined after the third day, until it reached background levels by the sixth day.

In Vivo Radionuclide Decorporation

When C2E5 was applied transdermally 24 h after the animals were contaminated with ^{241}Am using the wound contamination model (*i.m.* injection), the total ^{241}Am decorporation over a 7-day period was significantly enhanced when compared with untreated control animals (Table II). The enhanced decorporation showed a dose-dependent trend, with a nonsignificant increase in decorporation between 0 and 200 mg/kg dose, followed by significant increases in decorporation between the 200 mg/kg dose and the 600 mg/kg dose ($p < 0.05$) and between the 600 mg/kg dose and the 1,000 mg/kg dose ($p < 0.001$). The 1,000 mg/kg C2E5 dose achieved decorporation that was comparable to the current standard of care, *i.v.* DTPA. No statistically significant differences were found in the ^{241}Am contents recovered from the wound sites among all the experimental groups.

The observed increase in ^{241}Am decorporation was in part due to significant reductions in the ^{241}Am burden in the tissues of greatest concern, the liver, and skeleton. C2E5 significantly reduced liver burden compared with untreated animals at all the doses tested. A dose-dependent effect of C2E5 treatment was observed, with a decrease in mean liver ^{241}Am burden as the applied C2E5 dose increased; this effect was statistically significant when the 1,000 mg/kg dose was compared with the 200 and the 600 mg/kg doses ($p < 0.001$ and $p < 0.05$, respectively). The same trend was observed for the mean skeletal burden, with a significant reduction compared with untreated control animals at the 1,000 mg/kg dose. In all tissues examined, the effect of the highest transdermal C2E5 dose on ^{241}Am reduction was comparable with *i.v.* DTPA (Table II). The daily excretion of ^{241}Am in the urine (Fig. 3a) and feces (Fig. 3b) of untreated animals and animals treated with different doses of C2E5 further illustrated dose-dependent efficacy. Additionally, although the residual gel was removed from the skin 24 h after application (the same time that the day 2 urine and feces samples were collected), significantly enhanced decorporation was detected in the urine and feces at least 3 days after the gel was removed.

As transdermal drug flux is dependent on the drug application area, the decorporation efficacy of a C2E5 dose of 33.3 mg/kg/cm² applied to a 6 cm² versus an 18 cm² application area was determined. This threefold increase in application area resulted in significantly enhanced decorporation and a significantly reduced liver ^{241}Am burden compared with the untreated control ($p < 0.001$ and $p < 0.001$, respectively) and 200 mg/kg dose treatment groups

Fig. 1. Structures of C2E5 and its metabolites. For the study using radiolabeled C2E5, the C-14 label was on the carbonyl carbons of C2E5 and is retained on all C2E5 metabolites

($p < 0.001$ and $p < 0.05$, respectively) (Table II). The daily excretion of ^{241}Am in the urine (Fig. 3a) and feces (Fig. 3b) demonstrated that elevated ^{241}Am levels in the urine and feces were consistently achieved when the gel was applied to an 18-cm² area, compared to untreated controls as well as to animals that was treated with C2E5 doses of 200 and 600 mg/kg applied over a 6-cm² area.

DISCUSSION

Transdermal drug delivery possesses many advantages over other drug delivery routes such as parenteral and oral routes. These includes the delivery of a steady-state profile that reduces side effects related to fluctuations in plasma drug concentration, reduced dosing frequency, avoidance of first pass metabolism, and improved patient compliance due to its convenient and noninvasive means of self-administration (19,20,25). It may also offer benefits to special populations such as patients with needle phobia, those who are unconscious or too nauseated to take oral medications, pediatric patients, and the elderly. The latter two populations are specific areas of concern to the FDA related to the development of radionuclide decorporation agents (26). The stringent physicochemical requirements for potential transdermal drug candidates have limited the number of commercial transdermal drug products on the market (19,20). The DTPA prodrug, C2E5, was synthesized, determined to possess suitable physicochemical properties for transdermal delivery (21,22), and achieved sustained release of C2E5

metabolites when applied topically to rats in a nonaqueous gel formulation (23).

In the present study, the elimination profile for C2E5 was obtained, and dose- and area-dependent radionuclide decorporation efficacy profiles following transdermal application were demonstrated. The mass balance study using [^{14}C]-labeled C2E5 nonaqueous gels showed that approximately 62% of the applied C2E5 dose was absorbed in 24 h and that about half of the absorbed dose was eliminated in the urine. The overall [^{14}C] recovery for the study was 93%, a satisfactory end point for a mass balance study using rats, where at least 90% recovery is defined as acceptable (27). The small amount of [^{14}C] recovered from the skin application site indicated that C2E5 did not reside there for an extended period after administration, thus avoiding potential skin irritation and inflammation issues associated with drug retention at the application site. The fact that no signs of irritation were evident on visual inspection of the application site further confirmed the suitability of C2E5 for transdermal delivery. The daily excretion of [^{14}C] radioactivity in urine and feces (Fig. 2) showed sustained excretion of C2E5 and metabolites over 72 h following the removal of C2E5 gels from the application site. In addition, the recovery of most of the absorbed radioactivity in the excreta and the low residual radioactivity in the liver suggests that C2E5 and its metabolites were not retained in tissues.

We observed that about half of the absorbed C2E5 dose was eliminated in the urine over 6 days after topical administration. In contrast, i.v. DTPA results in $\geq 90\%$ renal clearance within 24 h of administration (12,13). C2E5 is metabolized by esterases in the skin and plasma in a stepwise manner, yielding metabolites such as DTPA, C2E1, and C2E2 (23); compounds that are more lipophilic than DTPA are known to shift elimination of actinides from a predominantly renal pattern to a pattern with increased fecal excretion (28,29). It is anticipated that these partially hydrolyzed C2E5 metabolites (C2E2 and C2E1) can form relatively stable chelating complexes with transuranic elements, such as ^{241}Am , with log stability constants in the range from 16.3 to 24.0 M⁻¹ (30,31). Even though such complexes are less stable than the ^{241}Am -DTPA complex, they may result in increased ^{241}Am decorporation consistent with the results observed in the C2E5 gel decorporation study. A strong temporal relationship was observed when the daily excretion of [^{14}C] in the urine is compared with the urinary

Table I. Percent of Administered Dose in Various Tissues 6 Days After Administration of [^{14}C]-Labeled C2E5 Nonaqueous Gels at a Dose of 200 mg C2E5/kg ($N=6$)

Sample	Mean	SD
Urine*	29.9	3.2
Feces	30.6	2.5
Skin at the application site	1.57	0.28
Liver	0.11	0.30
Recovered gel from the application site	30.8	6.6
Mass balance	93.0	3.9

*Include [^{14}C] recovered from cage washes

Fig. 2. Daily excretion of radioactivity in urine and feces after topical application of [^{14}C]-labeled 40% C2E5 nonaqueous gels (data are means $\pm$ S.D.) ($n=6$). (urinary excretion, $\blacksquare$; fecal excretion, $\bullet$)

fraction of the ^{241}Am decorporation. The relationship between fecal ^{241}Am decorporation and the content of [^{14}C] in the feces was less clear, possibly due to delayed biliary excretion of the chelation complex and intestinal transit time. These observations are consistent with earlier reports, where delayed excretion of transuranic radionuclides, such as ^{238}Pu , ^{239}Pu , and ^{241}Am , after chelation with DTPA or other decorporation agents was observed in dogs and rodents (17,28,32).

Animal models have been developed to facilitate the understanding of the biokinetic profiles that are observed following contamination by transuranic elements (14). For the model used in this study, a simulated wound contamination with [^{241}Am]-americium nitrate, the ^{241}Am at the intramuscular contamination site initially enters the bloodstream as the stable trivalent $^{241}\text{Am}^{3+}$ form. Once in the circulation, approximately 95% of the $^{241}\text{Am}^{3+}$ is cleared or redistributed from the plasma in less than 1 h, with the majority accumulating in the liver and skeleton (33–35). An adequate concentration of a chelator in the blood can sequester the $^{241}\text{Am}^{3+}$ as a stable chelation complex, reducing the translocation and deposition of radionuclides into the adjacent bone, liver, and other tissues. In addition to promote the excretion of $^{241}\text{Am}^{3+}$, Markley reported that intraperitoneally administered DTPA penta-ethyl ester successfully reduced the ^{239}Pu content in mouse liver (36). Guilmette *et al.* demonstrated that i.v. administration of a series of mono- and dialkyl esters of DTPA (including a form of C2E1 and C2E2, respectively) effectively reduced the plutonium burden in the skeleton of contaminated rodents (37). Because the biokinetics of ^{242}Cm and ^{244}Cm are very

similar to that for ^{241}Am (35), the effective decorporation treatment for ^{241}Am would also likely be effective in treating individuals contaminated with ^{242}Cm and ^{244}Cm . Thus, the previous reports suggest that this C2E5 gel treatment may promote the excretion of several transuranic elements in addition to ^{241}Am .

In the studies reported here, a well-defined C2E5 dose-dependent increase in excretion of ^{241}Am in the urine and feces was observed for animals treated with the C2E5 gels 24 h post contamination; total body decorporation of ^{241}Am increased by approximately 2% for every 100 mg/kg of administered C2E5. A similar trend was observed for the reduction in the liver ^{241}Am burden; each 100 mg/kg increment of C2E5 dose reduced the liver ^{241}Am burden by approximately 1%. Although the reduction in skeleton burden for 24 h post contamination C2E5 gel groups did not appear to be dose dependent, a lower skeletal burden was observed in contaminated animals treated with C2E5 gels at all dose levels compared to untreated animals. As the liver and bone tissues are key target organs for the chronic damage caused by radionuclide contamination (38,39), this improved ability for C2E5 metabolites to remove actinides fixed in liver and bone tissues may result in a therapeutic benefit. In addition to demonstrating a dose-dependent response to C2E5 treatment when applied to the same area, we also demonstrated increased efficacy when the application area was increased from 6 to 18 cm^2 . Based on Fick's law, the drug flux across the skin is directly proportional to the drug application area when all other parameters are kept constant. For animals treated with the C2E5 gel at a 1,000 mg/kg dose spread over

Table II. ^{241}Am Recovered in Samples 7 Days Following Single-Dose Treatments 24 h Post i.m. Contamination of Rats with ^{241}Am Nitrate (% of ID of ^{241}Am)

Treatment	Application area	Total excretion (urine + feces)	Liver	Skeleton	Wound site	Total recovery
Untreated control ($n=4$)	N/A	13.7 $\pm$ 2.6	23.6 $\pm$ 1.4	16.3 $\pm$ 1.3	30.2 $\pm$ 4.9	86.3 $\pm$ 3.4
i.v. Ca-DTPA 14 mg/kg ($n=8$)	N/A	37.7 $\pm$ 4.0***	12.1 $\pm$ 1.6***	11.7 $\pm$ 2.2*	24.7 $\pm$ 6.1	88.6 $\pm$ 2.1
C2E5 gel 200 mg/kg ($n=7$)	6 cm^2	18.8 $\pm$ 3.1###	19.2 $\pm$ 2.4***	14.0 $\pm$ 3.1	31.7 $\pm$ 7.5	87.9 $\pm$ 2.1
C2E5 gel 600 mg/kg ($n=8$)	6 cm^2	24.5 $\pm$ 3.2***###	16.2 $\pm$ 3.1***##	13.5 $\pm$ 1.8	30.4 $\pm$ 5.3	87.5 $\pm$ 1.7
C2E5 gel 1,000 mg/kg ($n=8$)	6 cm^2	33.3 $\pm$ 3.7***	13.1 $\pm$ 1.3***	12.5 $\pm$ 2.0*	23.0 $\pm$ 6.1	86.8 $\pm$ 2.1
C2E5 gel 600 mg/kg ($n=4$)	18 cm^2	32.7 $\pm$ 5.6***	14.6 $\pm$ 2.1***	12.9 $\pm$ 1.3	24.9 $\pm$ 7.5	88.5 $\pm$ 1.3

Significant difference by one-way ANOVA with Tukey's post hoc comparison of means, * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ against untreated control; # $p<0.05$, ## $p<0.01$, and ### $p<0.001$ against i.v. Ca-DTPA treatment. N/A not applicable

Fig. 3. Daily excretion of ^{241}Am in urine (a) or in feces (b) after a single dose of the decorporation agents at different dose levels and application areas 24 h post contamination. Significant (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) by Dunnett's test. (Untreated control, $\blacksquare$; 200 mg/kg applied to 6 cm^2 , $\bullet$; 600 mg/kg applied to 6 cm^2 , $\blacklozenge$; 1,000 mg/kg to 6 cm^2 , $\blacktriangle$; and 600 mg/kg to 18 cm^2 , $\blacksquare$)

6 cm^2 or at 600 mg/kg dose applied over 18 cm^2 , the overall efficacy in terms of enhancement in decorporation as well as reduction in liver and skeletal burden was comparable to that observed for animals intravenously administered Ca-DTPA at the standard recommended dose of 14 mg/kg.

All animals were examined daily during the experimental period for indications of disease or abnormalities including morbidity, mortality, and signs of toxicity (no radiotoxicity was anticipated due to the low amounts of ^{241}Am employed in these studies). In C2E5 treated animals, no elevated skin reddening or local skin inflammation was observed at the locus where the dose was applied, and gel treatment did not significantly alter animal body weights compared with untreated control animals. These preliminary results suggest that the C2E5 gel was well tolerated by the animals. The ^{241}Am content at the wound site for all the experimental groups was consistent with literature reports (14), and no statistical significance was observed in the retained ^{241}Am at wound sites among the different treatment groups. The radionuclide decorporation results confirmed the validity of maintaining chelator concentrations for an adequate duration to ensure optimal *in vivo* chelation of transuranic radionuclides (15).

Unlike most new drug candidates, the efficacy of radionuclide decorporation therapies has not been systemically evaluated

in human clinical trials due to ethical concerns involving the contamination of healthy human subjects with radionuclides. The "animal rule" (21 CFR 314.600) has been implemented by the FDA as a paradigm for the approval of drugs that treat radiological, chemical, and biological threats (40,41). The ^{241}Am decorporation results in the rodent wound contamination model presented here demonstrate that this treatment option can be as effective as the current FDA-approved i.v. DTPA treatment when administered 1 day after radionuclide contamination, a realistic response timeframe for victims in a mass casualty scenario after a nuclear/radiological event that has been adopted within the concept of operations by both the National Institute of Allergy and Infectious Diseases and the Biomedical Advanced Research and Development Authority.

In order to predict an appropriate human dosing regimen for this transdermal gel treatment based on rodent efficacy data, interspecies differences in the drug's pharmacokinetics need to be considered. Based on allometric scaling (42), the 6 cm^2 application area used on the ~ 250 g rats would translate to a gel application area of approximately 300 cm^2 (a circle with a radius of ~ 10 cm) for a 70 kg human; this application area is within the range of current topical products (43). The renal clearance of chelators like DTPA is slower in humans (1.3 mL/min/kg) than in the rat (5.9 mL/min/kg)

(44), which, for a given dose, would result in a higher steady-state plasma concentration in humans. It has also been demonstrated that the DTPA plasma concentration required to quantitatively bind ^{241}Am in rat plasma is approximately threefold greater than in human plasma (45). These two factors may result in a lower dose being required for human efficacy. The dose may be further lowered by increasing the frequency of dosing.

CONCLUSIONS

To our knowledge, this is the first report which demonstrates that the transdermal delivery of a pro-chelator is a viable strategy for delivering chelating agents to the systemic circulation, resulting in an effective, mass-casualty-ready treatment option for radionuclide contamination. Efficacy was demonstrated by enhancing total body decorporation and reducing the liver and skeletal burden of ^{241}Am with a single topical administration of the C2E5 gel 24 h after contamination. Enhanced ^{241}Am elimination for at least 3 days after application indicated that the C2E5 nonaqueous gels provided sustained delivery of metabolites capable of chelating ^{241}Am . No skin abnormalities or signs of skin irritation were observed after administration of the C2E5 nonaqueous gel throughout the entire study period. The effectiveness of this treatment option, favorable sustained release profile of pro-chelators, and ease of administration support the use of C2E5 nonaqueous gels following nuclear/radiological emergencies and for its inclusion in the Strategic National Stockpile.

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REFERENCES

- Benjamin GC, McGeary M, McCutchen SR, Editors; Committee on Medical Preparedness for a Terrorist Nuclear Event; Institute of Medicine. Assessing Medical Preparedness to Respond to a Terrorist Nuclear Event: Workshop Report. Washington, DC: The National Academies Press; 2009.
- International Commission on Radiological Protection. 1990 recommendations of the International Commission on Radiological Protection: user's edition. 1st Ed. Published for the International Commission on Radiological Protection by Pergamon Press, Oxford; New York; 1992.
- Wood R, Sharp C, Gourmelon P, Le Guen B, Stradling GN, *et al.* Decorporation treatment—medical overview. *Radiat Prot Dosim.* 2000;87(1):51–6.
- Harrison JD, Muirhead CR. Quantitative comparisons of cancer induction in humans by internally deposited radionuclides and external radiation. *Int J Radiat Biol.* 2003;79(1):1–13.
- Moysich KB, Menezes RJ, Michalek AM. Chernobyl-related ionizing radiation exposure and cancer risk: an epidemiological review. *Lancet Oncol.* 2002;3(5):269–79.
- Hameln Pharma GmbH. Package insert: pentetate calcium trisodium injection and pentetate zinc trisodium injection. Hameln Pharma GmbH; 2009.
- Guilmette RA, Moretti ES, Lindenbaum A. Toward an optimal DTPA therapy for decorporation of actinides: time–dose relationships for plutonium in the dog. *Radiat Res.* 1979;78(3):415–28.
- Taylor DM, Stradling GN, Hengé-Napoli M-H. The scientific background to decorporation. *Radiat Prot Dosim.* 2000;87(1):11–8.
- Stradling GN, Taylor DM, Hengé-Napoli M-H, Wood R, Silk TJ. Treatment for actinide-bearing industrial dusts and aerosols. *Radiat Prot Dosim.* 2000;87(1):41–50.
- Stradling GN, Hengé-Napoli M-H, Paquet F, Poncy J-L, Fritsch P, *et al.* Approaches for experimental evaluation of chelating agents. *Radiat Prot Dosim.* 2000;87(1):19–28.
- Stevens E, Rosoff B, Weiner M, Spencer H. Metabolism of the chelating agent diethylenetriamine pentaacetic acid (C14DTPA) in man. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, NY), Royal Society of Medicine. 1962; pp 235–238.
- Crawley FE, Haines JW. The dosimetry of carbon-14 labeled compounds: the metabolism of diethylenetriamine pentaacetic acid (DTPA) in the rat. *Int J Nucl Med Biol.* 1979;6(1):9–15.
- Phan G, Herbet A, Cholet S, Benech H, Deverre JR, *et al.* Pharmacokinetics of DTPA entrapped in conventional and long-circulating liposomes of different size for plutonium decorporation. *J Control Release.* 2005;110(1):177–88.
- National Council on Radiation Protection and Measurements. Scientific Committee 57-17 on Radionuclide Dosimetry Model for Wounds. NCRP Report No. 156, pp. 35–117. 2007.
- Ansoborlo E, Amekraz B, Moulin C, Moulin V, Taran F, *et al.* Review of actinide decorporation with chelating agents. *Cr Chim.* 2007;10(10–11):1010–9.
- Guilmette RA, Muggenburg BA. Reducing the radiation dose from inhaled americium-241 using continuously administered DTPA therapy. *Int J Radiat Biol Relat Stud Phys Chem Med.* 1988;53(2):261–71.
- Phan G, Le Gall B, Grillon G, Rouit E, Fouillit M, *et al.* Enhanced decorporation of plutonium by DTPA encapsulated in small PEG-coated liposomes. *Biochimie.* 2006;88(11):1843–9.
- Cassatt DR, Kaminski JM, Hatchett RJ, DiCarlo AL, Benjamin JM, *et al.* Medical countermeasures against nuclear threats: radionuclide decorporation agents. *Radiat Res.* 2008;170(4):540–8.
- Brown L, Langer R. Transdermal delivery of drugs. *Annu Rev Med.* 1988;39(1):221–9.
- Chien YW. Novel drug delivery systems. 2nd ed. New York: M. Dekker; 1992.
- Jay M, Mumper RJ. Methods and pharmaceutical compositions for decorporation of radioactive compounds. United States Patent 8,030,358; 2011.
- Sueda K, Sadgrove MP, Fitzsimmons JM, Jay M. Physicochemical characterization of a prodrug of a radionuclide decorporation agent for oral delivery. *J Pharm Sci.* 2012;101(8):2844–53.
- Zhang Y, Sadgrove MP, Sueda K, Yang Y-T, Pacyniak EK, *et al.* Nonaqueous gel for the transdermal delivery of a DTPA pentathyl ester prodrug. *AAPS J.* 2013;15(2):523–32.
- Volf V. Chelation therapy of incorporated plutonium-238 and americium-241: comparison of LICAM(C), DTPA, and DFOA in rats, hamsters, and mice. *Int J Radiat Biol Relat Stud Phys Chem Med.* 1986;49(3):449–62.
- Walters KA. Dermatological and transdermal formulations: Informal HealthCare; 2002.
- US Food and Drug Administration. Guidance for Industry, Internal Radioactive Contamination—Development of decorporation Agents. 2006.
- Roffey SJ, Obach RS, Gedge JI, Smith DA. What is the objective of the mass balance study? A retrospective analysis of data in animal and human excretion studies employing radiolabeled drugs. *Drug Metab Rev.* 2007;39(1):17–43.
- Lloyd RD, Bruenger FW, Mays CW, Atherton DR, Jones CW, *et al.* Removal of Pu and Am from beagles and mice by 3,4,3-LICAM(C) or 3,4,3-LICAM(S). *Radiat Res.* 1984;99(1):106–28.
- Bunin DI, Chang PY, Doppalapudi RS, Riccio ES, An D, *et al.* Dose-dependent efficacy and safety toxicology of hydroxypyridonate actinide decorporation agents in rodents: towards a safe and effective human dosing regimen. *Radiat Res.* 2013;179(2):171–82.
- Morss LR, Edelstein NM, Fuger J, Katz JJ. The chemistry of the actinide and transactinide elements, vol. 2. 3rd ed. Dordrecht: Springer; 2006. p. 1265–395.
- Sherry AD, Cacheris WP, Kuan KT. Stability constants for Gd^{3+} binding to model DTPA-conjugates and DTPA-proteins: implications for their use as magnetic resonance contrast agents. *Magn Reson Med.* 1988;8(2):180–90.

32. Sérandour AL, Grémy O, Fréchet M, Renault D, Poncy JL, *et al.* In vitro and in vivo assessment of plutonium speciation and decorporation in blood and target retention tissues after a systemic contamination followed by an early treatment with DTPA. *Radiat Res.* 2008;170(2):208–15.
33. Taylor D. Some aspects of the comparative metabolism of plutonium and americium in rats. *Health Phys.* 1962;8(6):673–7.
34. Turner G, Taylor D. The transport of plutonium, americium, and curium in the blood of rats. *Phys Med Biol.* 1968;13:535–46.
35. Ménétrier F, Taylor DM, Comte A. The biokinetics and radiotoxicology of curium: a comparison with americium. *Appl Radiat Isot.* 2008;66(5):632–47.
36. Markley JF. Removal of polymeric plutonium from mice by combined therapy with the calcium chelate and penta-ethyl ester of DTPA. *Int J Radiat Biol Relat Stud Phys Chem Med.* 1963;7:405–7.
37. Guilmette RA, Parks JE, Lindenbaum A. Synthesis and therapeutic testing of mono- and dialkyl esters of pentetic (diethylenetriaminepentaacetic) acid for decorporation of polymeric plutonium. *J Pharm Sci.* 1979;68(2):194–6.
38. Taylor GN, Lloyd RD, Mays CW, Angus W, Miller SC, *et al.* Plutonium- or americium-induced liver tumors and lesions in beagles. *Health Phys.* 1991;61(3):337–47.
39. International Commission on Radiological Protection. International Commission on Radiological Protection. Limits for intakes of radionuclides by workers. ICRP 30, Part 1. Ann. ICRP 2 (3-4). Oxford; New York: Pergamon Press; 1979.
40. Roberts R, McCune S. Animal studies in the development of medical countermeasures. *Clin Pharmacol Ther.* 2008;83(6):918–20.
41. Snoy PJ. Establishing efficacy of human products using animals: the US Food and Drug Administration's "Animal Rule". *Vet Pathol Online.* 2010;47(5):774–8.
42. Farriol M, Rosselló J, Schwartz S. Body surface area in Sprague-Dawley rats. *J Anim Physiol Anim Nutr.* 1997;77(1–5):61–5.
43. Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, *et al.* Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J Clin Endocrinol Metab.* 2004;89(5):2085–98.
44. Durbin P, Schmidt C. Predicting the kinetics of chelating agents in man from animal data. *Health Phys.* 1989;57:165–74.
45. Sueda K, Sadgrove MP, Jay M, Di Pasqua AJ. Species-dependent effective concentration of DTPA in plasma for chelation of ^{241}Am . *Health Phys.* 2013;105:208–14.