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DTPA TREATMENT OF WOUND CONTAMINATION IN RATS WITH AMERICIUM: EVALUATION OF URINARY PROFILES USING STATBIODIS SHOWS IMPORTANCE OF PROMPT ADMINISTRATION

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Abstract

INTRODUCTION: In the nuclear industry wound contamination with americium is expected to increase with decommissioning and waste management. Treatment of workers with diethylenetriaminepentaacetic acid (DTPA) requires optimization to reduce internal contamination and radiation exposure.

OBJECTIVES: To evaluate and compare different DTPA protocol efficacies after wound contamination of rats with americium.

METHODS: Wound contamination was simulated in rats by depositing americium nitrate in an incision in the hind limb. Different routes, times and frequencies of DTPA administration were evaluated. Individual daily urinary americium excretion and tissue retention were analyzed using the statistical tool STATBIODIS. Urinary profiles, urinary enhancement factors and inhibition percentages of tissue retention were calculated.

RESULTS: A single DTPA administration the day of contamination induced a rapid increase in americium urinary excretion that decreased exponentially over seven days indicating that the first DTPA administration should be delivered early as possible. DTPA treatment limited americium uptake in systemic tissues irrespective of the protocol. Liver and skeleton burdens were markedly reduced which would drive reduction of radiation dose. Local or intravenous injections were equally effective. Inherent difficulties in wound site activity measurements did not allow identification of a significant decorporating effect at the wound site. Repeated intravenous injections of DTPA also increased americium urinary excretion which supports the use of multiple DTPA administrations shortly after wound contamination.

CONCLUSION: Results from these statistical analyses will contribute to a better understanding of americium behavior in the presence or absence of DTPA, and may aid optimization of treatment for workers.

Keywords: contamination, internal; ^{241}Am; DTPA; analysis, statistical.

INTRODUCTION

The contamination of wounds is an important route of accidental radionuclide intake for nuclear industry workers, who may be injured by sharp contaminated objects at the work place (Piechowski et al. 2003, NCRP 2006, Grappin et al. 2007, Sugarman et al. 2018). The increasing decommissioning of nuclear facilities may put workers at greater risk of wound contamination given the use of various tools in a work environment involving mixtures of different radionuclides (Boice 2018). Contamination of wounds can result in a major entry of radionuclides in the body resulting from breach of tissue and vascular barriers (Ilyin 2001, Carbaugh et al. 2010). In this case, radionuclides may enter directly into the systemic circulation and become rapidly fixed by preferential tissues, e.g., liver and bones for actinides such as plutonium (Pu) or americium (Am) (ICRP 2019). Essentially all isotopes of Pu and Am are alpha emitters with emission energies of about 5 MeV and with long physical half-lives. When Pu and Am are retained in the body, sensitive tissues are exposed to alpha particles of high energy, which belong to the most detrimental category of radiation regarding living beings (ICRP 2007). A dose of radiation is consequently deposited and retained in the body, which can be quantified as committed effective dose (ICRP 2015). Prolonged exposure to these alpha emitters can increase the risk of developing stochastic effects in tissues, such as radiation-induced cancers. To maintain this risk “as low as reasonably achievable” (ALARA principle), treatments aim to remove incorporated radionuclides from the body and/or to avoid systemic transfer from the contamination site (Grappin et al. 2007, Avtandilashvili et al. 2018).

Treatment of wound contamination may include a surgical excision to remove most of the contaminated material (Poudel et al. 2018). For contamination with Pu or Am, diethylenetriaminepentaacetic acid (DTPA) can be administered locally and/or by intravenous (i.v.) injection (Grappin et al. 2007, Poudel et al. 2018, Sugarman et al. 2018). DTPA forms stable chelate complexes with Pu, Am and curium (Cm) which are excreted mostly in urine (Gremy et al. 2016).

DTPA treatment can reduce body retention of Pu or Am, and in consequence the committed effective dose (Davesne et al. 2016, Avtandilashvili et al. 2018). Both the U.S. Food and Drug Administration (U.S. FDA 2004) and the equivalent organization in France, "l'Agence Nationale de Sécurité des Médicaments et des Produits de Santé" (ANSM 2011) have approved DTPA for decorporation therapy after internal contamination with Pu, Am or Cm. DTPA can be applied locally as a rinsing solution after cleansing the wound site and/or during surgical resection. Intramuscular administration or injection directly into the wound site may be used but requires the addition of a local anesthetic such as procaine or lidocaine (1-2%) (Ilyin 2001, NCRP 2006). To prevent tissue uptake of Pu or Am, it is also recommended to administer an i.v. injection of DTPA as rapidly as possible after contamination. This is even more important in the case of wounds as radionuclides may enter the systemic circulation directly. Depending on the severity of the contamination, one or repeated i.v. injections of DTPA may be administered (Piechowski et al. 2003, Sugarman et al. 2018) . The dose administered should not exceed 1 g per day and dose by i.v. injection is generally 0.5 g, which represents a dose of 15-30 µmol/kg depending on body mass. The efficacy of DTPA is based mainly on increased urinary excretion of Am or Pu measured before and after treatment but it has been observed that DTPA-induced urinary enhancement ratios appear to be highly variable (Grappin et al. 2007, Davesne et al. 2016, Poudel et al. 2017). In French nuclear facilities DTPA is generally administered to a contaminated worker within a few hours after the incident. Therefore Pu or Am urinary excretion in the first 24 hours has already been influenced by the initial DTPA treatment and thus there are no time zero, i.e., without DTPA treatment data. In consequence it is difficult or even impossible to assess the actual increase of urinary excretion attributable to DTPA.

To understand better the behavior of actinides in the human body in absence or presence of DTPA and to optimize decorporation therapy depending on treatment parameters, animal experiments have been carried out previously using wound contamination models and chelation

therapy (NCRP 2006). Wound contamination has generally been simulated by subcutaneous (s.c.) or intramuscular (i.m.) injection in rats, to reproduce puncture wounds. Various treatment parameters have been explored, particularly for wound experiments using Pu: time of administration, route of administration (local i.m. injection, intravenous or intraperitoneal injection), mode and frequency of administration (bolus, infusion, single, repeated administration) and DTPA dose (Nenot et al. 1971a, Volf 1974, Stradling et al. 1993). The efficacy of actinide decorporation from the body by DTPA has been quantified by monitoring and comparing biodistribution data for untreated and treated groups of animals, i.e., reduced tissue retention and increased urinary excretion. Results were essentially reported as mean and standard deviation or error over groups of animals of small sample size.

The wound contamination models that have employed s.c. or i.m. injection induce little tissue damage and so do not reproduce the loss of vascular and tissue integrity that occurs after consequential tissue injury by wounding (Ilyin 2001, Carbaugh et al. 2010, Sugarman et al. 2018). Therefore, a more realistic experimental model in rats using deep incision of the hind limb was developed in the laboratory for contamination with Pu and/or Am (Griffiths et al. 2012). A similar model was also developed to simulate wound contamination with uranium, U (Houpert et al. 2004). In particular, this approach results in rupture of blood vessels so facilitating direct entry of the contaminant to the systemic circulation. This mimics a more complex situation than a s.c. or i.m. injection (Bistline et al. 1972, Piechowski et al. 1989). Using this model the efficacy of surgical excision and different DTPA administration protocols (single or repeated, and local or systemic) have been assessed on liver and skeleton retention and urinary excretion of Pu (Griffiths et al. 2014a, Griffiths et al. 2014b). Data analyses have been mostly descriptive and based on central values (means and standard deviations) over groups of animals with same contamination conditions and treatment regimens. The importance of the rapidity of DTPA administration shortly after contamination was not evaluated using this particular, more realistic wound model. Decorporation studies are required for Am as well as Pu following wound contamination for several reasons: (i) Am

is more soluble than Pu and behaves quite differently; (ii) exposure to Am may become increasingly prevalant after wound contamination with aged nuclear fuels during decommissioning procedures of old Pu nuclear facilities and in nuclear waste. It was therefore necessary to carry out additional experiments to evaluate the influence of the route and time of administration of DTPA on decorporation efficacy for Am. In this work, treatment regimens were especially designed to evaluate whether and to what extent DTPA can chelate Am at the wound site, in blood and/or in tissues as well as the importance of treatment time after wound contamination.

For over ten years the laboratory has generated biodistribution data after wound contamination with Am nitrate combined with different DTPA treatment regimens. In all cases, urinary excretion and tissue retention were measured. These biodistribution data obtained with numerous experimental conditions required a systematic approach for the statistical analysis and in particular to account for inter animal variability.

To facilitate the overall analysis of these large biodistribution datasets the statistical tool STATBIODIS was developed in the laboratory (Lamart et al. 2017, Lamart et al. 2019). It enables manipulation of a large set of individual data, adjustment of urinary excretion with sum of exponential terms, and comparison of data that accounts for small sample sizes, longitudinal data and mixed effects. STATBIODIS was used previously to analyse biodistribution data of Am obtained after wound contamination with Am nitrate, deposited alone or with Pu nitrate, or with MOX, with presence of Am as a decay product of ^{241}Pu (Lamart et al. 2019).

In this work, biodistribution data from wound experiments with Am have been collated and analysed to evaluate and compare the efficacy of various DTPA treatment regimens. These included variation in route, time and frequency of administration and experiments lasted from one to seven days. The evaluation of DTPA efficacy is illustrated by the comparison of untreated rats to rats treated with an i.v. injection of DTPA 2 h after contamination that was the reference protocol. The

effect of administration route and time elapsed between contamination and treatment on the decorporation efficacy of a single DTPA administration have been subsequently estimated. Finally, the efficacy of single or repeated i.v. injections of DTPA doses is compared.

MATERIAL AND METHODS

Experimental data

Individual biodistribution data

Individual tissue retention and urinary excretion data were collated from experiments carried out to evaluate the efficacy of different DTPA treatment protocols after wound contamination with Am nitrate. Available data were obtained from experiments carried out over a period of eight years (Table 1). All experiments were in compliance with the European and French regulations for animal experimentation (European directive 2010/63/EU, September 22, 2010 and French decree 2013-118, February 1, 2013) and were approved by the local animal ethics committee.

Male Sprague Dawley rats weighing 300–500 g were used for all experiments. Wound contamination was carried out under deep anesthesia (sodium pentobarbital 50 mg/kg, i.p.) after incision of the left hind leg and a deposit of an Am nitrate solution with an activity of approximately 5 kBq in 50 µL ($AmNO_3$; 99.4% of ^{241}Am in activity) (Griffiths et al. 2012). Aliquots of the Am contamination solution were sampled regularly during the experiments and measured by liquid scintillation counting to assess the deposited activity. After contamination the wound was sutured and the animals allowed to recover. The contamination site was gently cleaned using a “nasal wipe”, to remove local, external Am. The activity at the wound site was measured (i) for most experiments at the time of contamination on the whole animal whilst under anesthesia and considered as the administered activity, and (ii) after euthanasia on the contaminated leg for all experiments. Direct counting of the wound site on the anesthetized animal after contamination has recently been suspended to promote better radiation protection conditions. More recent determination of administered activity was carried out by subtracting the activity removed using the nasal wipe from the theoretical deposited activity measured as described above in the aliquots of the contamination

solution. The activities assessed using both methods showed a satisfactory agreement. Animals were housed in metabolism cages for collection of 24 h-urines samples for up to seven days. Up to five systemic tissues were collected at the time of euthanasia (kidney, liver and femur; blood and lymph nodes for some experiments). Activity in some samples was measured by gamma spectrometry using a NaI(Tl) scintillation detector or a germanium detector (contamination site, nasal wipe, urines). Tissue samples were dry ashed (500-600°C depending on sample) and wet ashed in HNO_3 (2 mol/L) and H_2O_2 (30%) and activity measured using liquid scintillation counting. In some cases and generally for low activities, urine samples were wet ashed and treated as described above. Bone retention was obtained by multiplying femur activity by a factor of 20 (Griffiths et al. 2012). Uncertainties have been evaluated at 30% (k=2) for samples measured by liquid scintillation counting from the mineralisation step onwards and at 20% for gamma-spectrometry. The retention and excretion data were obtained from multiple experimental steps - from the preparation of the contamination solution to the activity measurement in biological samples. Thus the variability observed between the data may be explained in part by the resulting overall uncertainties inherent in the measurements.

To evaluate different decorporation protocols, diethylenetriaminepentaacetic acid (DTPA) was injected intravenously into the tail vein or locally at the wound site, at a dose of 30 µmol/kg, which corresponds to the maximal dose used in man, and at three times after contamination 30 min, 2 h or 1 day (Table 1). The effect of repeated administrations was also assessed with subsequent i.v. injections at 30 min, 1, 2 and 3 days.

Statistical analysis with STATBIODIS

Experimental data from the wound experiments were analyzed using the statistical tool STATBIODIS, based on the R language (Lamart et al. 2017, Lamart et al. 2019, R Core Team 2019).

Formatting treatment data and selecting data for the analysis

The standard format used to organize and archive the raw data in STATBIODOS was extended from the original in order to incorporate treatment data (Lamart et al. 2017). For groups of rats that received DTPA treatment, two additional tables saved in CSV files (CSV: Comma Separated Values) were created to code for treatment parameters. The first one included treatment drug, route, frequency and dose, e.g., DTPA, local, single, 30 µmol/kg, respectively. The second one contained treatment times in days. All data related to Table 1 were therefore organized according to this updated format.

Individual raw data from each rat were processed using STATBIODIS for computation of new variables, individual data visualization and descriptive analysis. For example, tissue retention and urinary excretion data were calculated as percent of the administered activity for use in the subsequent steps of the analysis.

As the number of experiments has increased in STATBIODIS, a function was developed to select the individual data from the whole database based on experimental characteristics. This function reads the whole database and selects the matching data corresponding to the characteristics specified for the selection, e.g., element (Am), physico-chemical form (Nitrate), time of euthanasia (1 or 7 days). Additional characteristics can be easily added for a particular selection.

Evaluation, quantification of treatment efficacy and comparison of DTPA treatment protocols from analysis of individual biodistribution data

The efficacy of DTPA treatment was evaluated and quantified for Am urinary excretion and tissue retention by comparing data between untreated and treated groups of rats. An i.v. injection of DTPA administered 2 h after wound contamination was used as reference.

When a significative effect of treatment was obtained, the DTPA treatment efficacy was quantified for each treated rat as the urinary excretion enhancement factor and as the inhibition percentage of tissue retention (Equation 1 and Equation 2, respectively):

$$Enhancement_i^{treated}(t) = \frac{Excretion_i^{treated}(t)}{Excretion_{MEDIAN}^{untreated}(t)}$$

Equation 1

Where $Excretion_i^{treated}(t)$ is the 24 h urinary excretion at time t of the treated rat i and $Excretion_{MEDIAN}^{untreated}(t)$ is the median value for the untreated group.

$$Inhibition_i^{treated}(t) = \frac{Retention_i^{treated}(t) - Retention_{MEDIAN}^{untreated}(t)}{Retention_{MEDIAN}^{untreated}(t)} \times 100$$

Equation 2

Where $Retention_i^{treated}(t)$ is the tissue retention at time t of the treated rat i and $Retention_{MEDIAN}^{untreated}(t)$ the median value for the untreated group.

Several R functions were developed to automate the computation of the urinary enhancement factor and inhibition percentage of tissue retention for each treated rat and at each time point.

To evaluate the influence of DTPA treatment on the relative systemic tissue distribution, ratios of activity between tissues with the highest Am retained activities were calculated and compared (skeleton to liver, kidney to liver and kidney to skeleton).

To compare the efficacy of the different DTPA treatment protocols, the effect of treatment time and route were evaluated separately, using data obtained after treatment at 30 min, 2 h or 1 day; and after i.v. or local administration, respectively. For rats treated at 1 day after contamination,

the ratio of daily urinary excretion between day 2 and day 1 was computed to assess the influence of the treatment, with each rat being its own control. In addition, the effect of treatment regimen was evaluated by comparing a single i.v. administration of DTPA at 30 min with a repeated administration at 30 min, 1, 2 and 3 days after contamination. Comparisons were carried out for all time points, i.e., from one to seven days for daily Am urinary excretion; and for one or seven days for tissue retention.

Comparison tests

Prior to running comparison tests, data and effects of interest were identified and characterized in terms of sample size, paired or unpaired data, fixed or random effects. Non parametric rank-based methods adapted to small and uneven samples, independent or paired data, and mixed effects were applied (Lamart et al. 2019). Overall effects were evaluated using f1.LD.f1 function from the {nparLD} R package (Noguchi et al. 2012). When the outcome was significant, pair comparisons were carried out using the Wilcoxon test. The Wilcoxon.test function from R provides a p-value and a pseudo-median difference between independent data (Bauer 1972). The Bonferroni or Holm correction was applied to the obtained p-values depending on sample size. Significant levels of 0.05 and 0.025 were chosen for bilateral and unilateral tests, respectively.

To facilitate the increasing use of pair comparison tests, a function was developed to automate the application of the Wilcoxon test along longitudinal data. This function can handle unilateral or bilateral tests between pairs of data derived from two groups of rats associated with different contamination or treatment characteristics, e.g. treatment time at 2 h versus 1 day after contamination. The function runs the comparison for each pair along the values associated with longitudinal data, e.g., time for daily Am urinary excretion from day 1 to 7. The inputs and results of the comparison are automatically exported to a table and saved as a CSV file for interpretation.

Urinary excretion function

To quantify the variation of urinary excretion kinetics with treatment, urinary excretion data were adjusted with a sum of exponential terms for untreated and treated rats, respectively, and according to Equation 3. Fitted curves were obtained using a non-linear regression accounting for the time after contamination as a longitudinal variable and the rat as a random variable. The R function nlmer of the {lme4} R package was applied in STATBIODIS with (restricted) maximum likelihood (Bates et al. 2015).

$$f(t) = \sum_{i=1}^{n} a_i e^{-r_i t}$$

Equation 3

With t the time in days and a_i (% of administered activity) and r_i (/day) the regression coefficients.

RESULTS

Evaluation and quantification of treatment efficacy: application to a single i.v. injection of DTPA 2 h after contamination

This initial section explains how treatment efficacies were evaluated and quantified using two particular groups of animals. For illustration individual urinary Am excretion profiles and tissue retention data were compared between untreated rats and rats treated with an i.v. injection of DTPA 2 h after contamination.

Daily urinary Am excretion from one to seven days

- ***Is there an effect of the DTPA treatment on urinary excretion of Am and at which time after contamination?***

Daily urinary Am excretion from one to seven days for each rat was compared between untreated and treated groups (Figure 1). For untreated rats, urinary Am excretion during the first 24 h after contamination represented 1%-10% of administered Am activity as compared with 12%-30% for DTPA-treated rats. Daily Am excretion decreased with time in both groups. From day two onwards values were less than 2% for untreated rats and 8% for treated rats.

To select the appropriate statistical tests, the characteristics of the data and effects were identified. An important first observation was that interindividual variability in Am urinary excretion was important and that it could not be considered as a controlled parameter (Figure 1). Therefore the animal was considered as a random variable. Data obtained from untreated and treated rats were independent and treatment corresponded to a fixed effect (Table 2). As urinary Am excretion was measured repeatedly for each animal from one to seven days the data obtained over time formed paired (or longitudinal) data.

The overall effects on 24 h urinary Am excretion were evaluated statistically accounting for rats as a random variable, i.e., the effect of treatment with all times considered, the effect of time after contamination regardless of treatment, and the effect of the interaction between treatment and time (Table 2). All effects were found to be significant. In particular, the effect of the interaction between treatment and time indicated that the effect of treatment depended on the time after contamination. Hence, multiple comparisons of urinary Am excretion data were carried out at each time point between untreated and treated groups. As DTPA was expected to increase urinary excretion, it was assessed whether urinary excretion was greater in treated rats than in untreated rats (unilateral alternative hypothesis H1, Table 3). Following treatment, Am excretion was significantly greater than in untreated rats at all times (1 to 7 days), with pseudo-median differences in Am activity ranging respectively from 19% to 0.6% of the initial administered activity.

The data clearly demonstrate that a single i.v. injection of DTPA 2 h after wound contamination increased urinary Am excretion and that the effect remained significant for up to at least seven days.

- ***Quantification of the individual enhancement factor in urinary Am excretion following a single DTPA treatment***

The enhancement factor of 24 h urinary Am excretion was calculated for each treated rat at each time after contamination using the median data from the untreated group (Equation 1, Figure 2). Due to the variability between treated rats, this factor varied from 3 to 13 overall, with medians per time point ranging from 5 to 11 between one and seven days.

Although the difference in excreted activity was much greater for example for day 1 than for day 7 (19% and 0.6% of the administered activity, respectively; Table 3), the enhancement factor for urinary Am excretion resulting from a single i.v. dose of DTPA remained in the same range between day 1 and 7 (Figure 2). .

This lasting effect of DTPA may result from the large excess of DTPA at the time of administration as compared with the Am activity deposited in the wound. An activity of 5 kBq of Am used for wound contamination corresponded to 1.63 x 10^{-10} moles of Am, whereas an injection of DTPA as 30 µmol/kg in a rat weighing 400 g would correspond to 1.20 x 10^{-05} moles of DTPA. As one molecule of DTPA can chelate one Am ion, there would be a 70000-fold excess of DTPA at the time of administration. Although DTPA half-life in the body was estimated to be less than 24 h (Stevens et al. 1978, Stather et al. 1983, Fritsch et al. 2010), there would be sufficient molecules of DTPA remaining in the body beyond day 1 to chelate Am.

- ***Fit of exponentials to urinary data***

The 24 h urinary excretion of Am was fitted using non-linear regression with a sum of two exponential terms for untreated and treated rats (Table 4). Regression coefficients for untreated groups, *e.g.*, $r_1 = 3.7 \pm 0.2\ d^{-1}$ and $r_2 = 0.3 \pm 0.03\ d^{-1}$, remained similar to those published with wound contamination data obtained up until 2016, $r_1 = 3.6 \pm 0.2\ d^{-1}$ and $r_2 = 0.3 \pm 0.03\ d^{-1}$ (Lamart et al. 2019). This observation confirmed that recent wound experiments were consistent with those carried out previously.

Regression coefficients a_1 and a_2 were respectively 4 and 11 times greater for data obtained from DTPA-treated animals than without, whereas r_1 and r_2 coefficients varied respectively by -3% and +33% with treatment. This result confirmed that Am urinary excretion after DTPA treatment was significantly greater than for untreated group. In addition, despite greater Am activities in urine, the excretion profile remained similar. This indicates that the kinetics of urinary Am excretion would be the same regardless of the form in which Am is excreted, i.e., chelated to DTPA or not.

As DTPA was administered in a large excess as compared with Am, it would be reasonable to assume that every available ion of Am was chelated by DTPA and that Am excreted in urine of treated rats should mostly be in the form of an Am-DTPA chelate. Therefore, the Am urinary

excretion obtained in untreated rats represented the excretion of Am complexed to citrate or a protein, whereas the Am urinary excretion in treated rats represented mostly the capture and elimination of Am complexed to DTPA.

In addition to the exponential curves adjusted for untreated and treated groups respectively, the urinary excretion for each rat was also fitted for the overall non-linear regression analysis since the rat is considered as a random variable. The values predicted by each fitted curve were in good agreement with measured activity in daily urine samples of each rat (Supplemental figure 1 and 2 for untreated and treated rat, respectively). The overall fit associated to each treatment group accounted for the variability between animals and the individual regression curves.

Am retention in tissues

- ***Is there an effect of the DTPA treatment, on which tissue and at what time after contamination?***

After wound contamination with Am, a fraction is transferred to blood and subsequently to systemic tissues with a possible uptake and retention of the actinide. The administration of DTPA 2 h after contamination may remove activity from wound and so prevent deposition in tissues. The individual tissue retention data obtained at day 1 and 7 were compared between untreated rats and rats treated with an i.v. injection of DTPA 2 h after wound contamination with Am nitrate (Figure 3 and Supplemental Table 1). Treated groups belonged to single experiments with times of euthanasia at 1 and 7 days respectively, and presented less variability than for untreated groups.

Systemic tissues are discussed first. Regardless of treatment, Am activity was mostly retained in liver, skeleton and kidney, with 5%-25% of Am administered activity in liver for untreated rats and 2%-5% in treated rats.

The overall effects of treatment, tissue and the interaction of time and tissue, as considered from a statistical point of view, were evaluated at each time after contamination (Supplemental Table 2). At both times, there was an effect of treatment and an effect of the interaction between tissue and treatment, i.e., the magnitude or direction of the treatment effect changed with tissue. Multiple comparisons were therefore carried out to test whether tissue retention was reduced after DTPA treatment (unilateral alternative hypothesis, H1; Table 5). At both times after contamination, Am retention was found to be significantly less in kidneys, liver and skeleton in treated rats as compared with untreated, with pseudo-median differences in Am activity of -1%, -9%, and -16% of Am administered activity, respectively at day 7.

Although the test for retention at contamination site did not attain statistical significance at day 1, the test suggested some detectable difference at day 7 with a p-value close to the significance level (0.027). There may well be a reduction of wound retention in the treated rats, but it may be too small to be measured and so difficult to identify. Indeed, wound measurement remained uncertain given that the Am depth and lateral distribution at the wound site was not well-known and variable between animals.

In summary, despite the variability observed for untreated group, there was a decorporating effect of the i.v. DTPA injection 2 h after contamination on systemic tissue retention, which was observed both at day one and seven. Therefore this early injection of DTPA limited the uptake of Am in tissues.

- ***Influence the time of tissue activity analyses after contamination***

Treatment effect on tissue retention may differ with time after contamination. To address this question, retention data were compared between day 1 and 7 for untreated and treated rats, respectively (Figure 3).

For untreated rats, there was an overall effect of the interaction between time and tissue on tissue retention ($p=2 \times 10^{-10}$; Supplemental Table 3). For treated rats, there was an effect of the time after contamination ($p=2 \times 10^{-9}$). Multiple comparisons were carried out to evaluate whether tissue retention data were different between day 1 and 7 for untreated and treated rats separately (bilateral alternative hypothesis, H1; Supplemental Table 4). For untreated rats, a significant difference was observed for blood levels with a pseudo median difference of only 0.05% (95% CI: 0.03% to 0.08%), and a tendency for bone levels with p-value close to the significance level (0.06). For treated rats, tissue retention was significantly different between day 1 and 7, except for skeleton. Still, this difference was quite moderate as the p-values were relatively high (0.03) and the pseudo-median differences in tissue Am activity were small as compared with the variability between animals.

For untreated rats, there were no major differences between day 1 and 7 either in systemic tissue or in wound retention indicating that most of the transfer of Am from wound to blood and subsequent deposition in systemic tissues had already occurred at day 1, i.e., within the first 24 h after wound contamination. In untreated rats, excreted Am activity corresponded mainly to a fraction of Am that is readily transferable at the time of contamination. One day after contamination, there was hardly any Am circulating in blood. Therefore, Am had been either excreted or retained mainly in liver and skeleton.

After DTPA treatment 2 h after contamination, systemic tissue retention (except for skeleton) was slightly less at day 7 than at day 1. Overall these observations indicated that:

- Most of DTPA action occurred within the first 24 h as soon as Am was transferred to blood, i.e., DTPA administration prevented deposition in systemic tissues of the fraction of Am available for chelation.

- There was some additional action of DTPA between day 1 and 7. Although most of DTPA would be excreted from the body within 24 h (Nenot et al. 1971b, Stevens et al. 1978), the remaining fraction of DTPA was sufficient to chelate Am further where accessible. However, the effect of DTPA could not be observed by comparing the skeleton retention between day 1 and 7, indicating that Am retained in skeleton may not become available to DTPA over this time-period.

The retention of Am measured at the wound site was highly variable among untreated animals (Figure 3). No difference in wound retention could be identified between day 1 and 7 (p=0.06; Supplemental Table 4). On the contrary, wound measurement results were well-grouped for treated rats (Figure 3). A significative difference was found between day 1 and 7, though with a relatively high p-value (0.03), and with 26% less retention at day 7 than at day 1. An additional effect of DTPA between day 1 and 7 may be explained by a greater solubilization of Am at wound site by DTPA. However, data obtained for treated rats at day 1 and 7 were derived from two separate experiments respectively, sample sizes were small, and the determination of wound retention was more difficult and uncertain than for tissues. Therefore, DTPA efficacy on wound retention and the associated effect size could be further evaluated and confirmed by additional experiments.

Overall, the moderate decrease identified in Am tissue retention between day 1 and day 7 for treated rats would be consistent with the lasting increase of excretion described above.

- ***Individual inhibition percentage of retention in tissue***

The individual inhibition percentage of retention in tissue was calculated for rats treated with an i.v. injection of DTPA 2 h after wound contamination. Only tissues for which a significant treatment effect was observed are presented for both 1 and 7 days after contamination (Equation 2, Figure 4). At day 7, inhibition ranged from about -60% to -80%, -70% to -80%, and -40% to -70% % in kidneys, liver, and skeleton, respectively. Inhibition was thus in the same order of magnitude for

kidneys as for liver and skeleton (Figure 4), whereas kidney retention was much smaller (Figure 3). Therefore DTPA administration 2 h after contamination resulted in less Am activity available for overall systemic deposition, with an indication of a relatively greater inhibition in liver than in skeleton. Moreover inhibition appeared greater at day 7 than at day 1 in the three tissues, which was consistent with the lasting effect of DTPA previously observed between day 1 and 7.

Tissue activity ratios

To evaluate the influence of treatment on relative tissue distribution, the ratio of Am activity between the main retention tissues was compared between untreated rats and rats treated with i.v. DTPA 2 h after contamination at day 1 and 7 (Figure 5 and Supplemental Table 5). At day 7, the skeleton to liver activity ratio ranged from 1-3 for untreated rats and from 4-6 in treated rats.

At both times after contamination, there was an overall effect of treatment, tissue ratio and of the interaction between treatment and tissue ratio (Supplemental Table 6). Multiple comparisons between untreated and treated rats resulted in a significant difference for skeleton to liver ratios at both times after contamination (Supplemental Table 7). Also, a greater pseudo-median difference in the skeleton to liver ratio was obtained at day 7 than at day 1: -2.4 (95% CI: -3.1 to -1.7) and -0.7 (95% CI: -1.2 to -0.3), respectively. These results were coherent with previous observations indicating a greater efficacy of DTPA to attenuate Am retention in liver as compared with the skeleton. Moreover this differential effect between liver and skeleton seemed to increase with time. Am may be more accessible to DTPA in liver than in skeleton between day 1 and 7.

Comparison of Am biodistribution data between different treatment times and routes for a single DTPA administration

The previous section highlighted the effect of a single DTPA treatment by comparing untreated and treated groups of animals. The following addresses the influence of treatment time and route. Using the same approach as above, the individual 24 h urinary excretion of Am was compared between three DTPA treatment times (30 min, 2 h and 1 day) and for two routes of DTPA administration (i.v. or local; Figure 6). Similarly, tissue retention was compared between these different treatment protocols at day 1 and 7 (Figure 7 and Supplemental Table 10).

Comparison between treatment times

- ***Daily Am urinary excretion***

With regard to animals treated at day 1 after contamination 24 h urine samples were collected prior to DTPA administration (see data inside the box on Figure 6). Am excretion data were therefore expected to be comparable to those obtained previously for untreated rats at day 1.

The estimation of overall effects for each treatment route showed that there was an effect of the interaction between treatment time and time of urine collection after contamination (Figure 6, Supplemental Table 8). Multiple comparisons were carried out between treatment times, using time of 2 h as a reference. There were no differences in daily urinary Am excretion from day 1 to 7 for DTPA administration at 30 min and 2 h and irrespective of treatment route. There were also no differences in Am excretion from day 2 to 7 after contamination for an early (2 h) or delayed (1 day) DTPA administration. Therefore, when the administration is delayed for 1 day less activity is available for chelation and so the effect on urinary Am excretion is much less marked than that observed following DTPA administration at early times (30 min, 2 h).

Using urinary Am excretion of rats treated at day 1, it was possible to compute the ratio of Am excretion before and after DTPA administration, i.e., between day 1 and 2 (Supplemental Figure 3). When data are available, this ratio is computed similarly for human cases of contamination. For treatment time at day 1, this ratio ranged from 2-4, whereas for treatment time at 2 h, the urinary enhancement factor computed between untreated and treated rats varied from 3-8 at day 1 (Figure 2). Results are not directly comparable as (i) the method of computation was different between the ratio and the enhancement factor and (ii) treatment times changed too (2 h versus 1 day).

- ***Am retention in tissues at day 1 and 7***

For each treatment route and time of euthanasia, the estimation of overall effects showed significance for treatment time and/or interaction between treatment time and tissue, except for local administration with euthanasia at day 1 (Figure 7; Supplemental Table 11).

Multiple comparisons between treatment times with i.v. injection of DTPA at 2 h taken as the reference showed that there were no differences in tissue retention for each DTPA administration route and time of sample collection after contamination (Supplemental Table 12 and Supplemental Table 13). Following DTPA administration at day 1, 20 % less of urinary Am activity was recovered as compared with the earlier administration (30 min or 2 h) where much of the activity was excreted during the first 24 h after contamination. It was therefore assumed that the corresponding missing activity would be retained in the body, at the wound site and/or in systemic tissues.

Comparison of treatment routes

The interindividual variation was greater within the local DTPA treatment group as compared to the i.v. treatment group (Figure 6). This observation may be explained by differences in the access and rapidity of DTPA distribution in the body between the two routes of administration. An

intravenous bolus injection results in high DTPA levels immediately in the systemic circulation. The local delivery of DTPA into the wound site is likely to be more variable and will depend on the wound, the degree of vascular and tissue lesions and on the position of the injection needle. At 2 h after contamination wound-healing processes such as influx of acute phase proteins including transferrin could also play a role in the accessibility of DTPA into the circulation. There is of course much less variation in an i.v. injection for treatment administration.

For each treatment time, no difference in Am urine excretion were found between treatment routes (Supplemental Table 14 and Supplemental Table 15). Likewise, there were no major differences in tissue retention whatever the time of treatment or of euthanasia. Indeed differences were negligeable as compared with variability between individuals (Supplemental Table 16 and Supplemental Table 17).

Therefore both treatment routes had an effect on systemic tissue retention and this effect was of the same order of magnitude. As external measurement of the wound site was challenging no significant effect on the contamination site could be demonstrated.

Comparison of a single and a repeated treatment

The previous section compared different treatment times and routes of a single administration of DTPA. In this section, a single i.v. injection of DTPA 30 min after wound contamination at a dose of 30 µmol/kg was compared to a repeated DTPA i.v. injection at the same dose administered at at 30 min, 1, 2 and 3 days. The total dose of DTPA was four times greater.

Daily Am urinary excretion

The individual urinary excretion of Am was compared between single and repeated i.v. injections at 30 min, 1, 2 and 3 days (Figure 8). Overall effects were estimated significant for treatment frequency, time of urine collection after contamination and the interaction of frequency and time (Supplemental Table 18). Multiple comparisons of 24 h urine data showed that the repeated treatment resulted in greater urine excretion from day 2 to 6 with a maximum increase of excretion at day 3 with a pseudo-median difference of 4.2% as compared with a single i.v. injection at 30 min (Table 6). The increase of excretion from day 2 obtained between the repeated i.v. injection and a single i.v. injection remained moderate and was of the same order of magnitude of that obtained from day 2 between a single i.v. injection at 2 h and no treatment.

As DTPA was administered in a large excess at the first i.v. injection, the fraction remaining in the following days was presumably adequate to explain the maintained effect after this single administration. Subsequent i.v. injections of DTPA administered after the first i.v. injection would therefore have a supplemental but lesser effect than the first i.v. injection. This result may suggest that it is beneficial to repeat DTPA administration shortly after contamination with Am nitrate but to decrease the frequency of administration to every other day as opposed to daily dosing. Clearly this would be better for patient well-being and compliance.

Am retention in tissues

Individual retention data were also compared between treatment frequencies (Figure 9, Supplemental Table **19**). As the overall effects were significant (Supplemental Table 20), multiple comparisons were carried out to evaluate whether tissue retention was further reduced after a repeated treatment than after a single treatment (Supplemental Table 21). When considered separately, effects were not significant.

In summary, although there was a greater urinary Am excretion after a repeated DTPA treatment as compared with a single treatment, no significant further decrease in tissue retention was observed. The increase in excreted activity might have come from other tissues and/or from the wound but could not be identified.

DISCUSSION

In this work, the efficacy of different DTPA protocols after wound contamination in rats with Am nitrate were evaluated and quantified based on individual urinary Am excretion profiles and tissue retention of Am. The data analysis also provided (i) insights for interpretation of human data, (ii) recommendations on treatment regimes and (iii) additional information for the development of biokinetic models that address DTPA efficacy.

The statistical analysis using STATBIODIS enables the handling of a large variety of contamination and treatment parameters both numerically and graphically. Adapted statistical methods were systematically applied to individual biodistribution data to account for small sample sizes, variability between rats, paired data and mixed effects. Although comparison of results across experiments showed satisfying coherence, remaining variability between experiments may reduce the ability to identify effects, when effect size was small. The current approach could be improved by accounting for the animal and the experiment both as random variables, and/or by applying the pointwise comparison method carried out by Mezaguer and colleagues using the gls function of the {nlme} R package (Mezaguer-Lekouaghet et al. 2019). Most importantly, the development and use of STATBIODIS fulfils the application of the 3 Rs rule (Replacement, Reduction and Refinement) of the ethical principles in animal research, in radiation toxicology (Griffiths et al. 2020).

The efficacy of an i.v. DTPA injection administered at 2 h after wound contamination with Am nitrate was evaluated and quantified based on individual daily urinary Am excretion and tissue retention of Am. Where a significant effect of DTPA was observed, three complementary methods were used to quantify treatment efficacy: (i) the pseudo-median difference between untreated and treated groups, expressed as percentage of activity or activity ratio, (ii) the urinary enhancement

factor or inhibition percentage of tissue retention, and (iii) the adjustement of urinary profiles for untreated and treated groups.

By comparing daily urinary Am excretion from 1 to 7 days between untreated and treated rats, this work showed a lasting effect of a single i.v. administration of DTPA after wound contamination, with a stable urinary enhancement factor. This observation was in line with those obtained for other experimental models of contamination with Pu and Am, e.g., with injection (Gremy et al. 2016) and inhalation (Fritsch et al. 2009). Furthermore, the computation of the urinary enhancement factor indicated that the decorporation efficacy remained in the same order of magnitude for seven days after contamination.

An interesting contribution of the present work concerns the urinary Am excretion profiles of untreated rats and rats treated with a single i.v. DTPA injection at 2 h after contamination. The fitted exponential curves to urinary excretion of Am could be useful in the interpretation of human urinary data after DTPA treatment because: (i) daily urinary data of the worker is rarely collected for seven consecutive days after a prompt and single DTPA administration, (ii) as soon as DTPA treatment has begun, the amount of activity that would have been excreted by the worker without treatment is difficult to determine given the lasting effect of DTPA. This excretion profile represents a model of the prompt enhancement of excretion obtained for rats with DTPA, but also of the lasting effect of a single injection shortly after contamination. These excretion models could be used to evaluate the actual urinary enhancement factor obtained with DTPA. Furthermore, these could serve as entry data in the development of a biokinetic model of Am after DTPA treatment for wound contamination, as the urinary excretion curve obtained for treated rats represents the kinetics of capture and elimination of Am by DTPA (Kastl et al. 2014, Poudel et al. 2017, Dumit et al. 2018, Miller et al. 2018).

This work also showed that a prompt administration of DTPA diminished Am retention in liver, skeleton and kidneys, by limiting the Am uptake in tissue. Although a single DTPA administration was efficient in reducing retention in these three tissues, DTPA efficacy appeared better for liver than skeleton. There might have been little bone remodeling within the first week after contamination a factor that would permit better access of DTPA to bone-associated Am. As liver and skeleton retain the largest amounts of activity within systemic tissues, the averted radiation dose for workers is expected to depend greatly on the reduction of activity in these two tissues. The pseudo-median differences of Am activity obtained in this work between treated and untreated groups (e.g., -9% and -16% for liver and skeleton at day 7, respectively) provided an estimate of this reduction.

The quantification of DTPA efficacy following actinide contamination reported by previous decorporation studies were difficult to compare with that of the present work because wound contamination models, treatment protocols, and time of euthanasia varied greatly across studies. In addition there were fewer data for Am than for Pu. However other authors have reported that DTPA administration resulted in a reduction of tissue retention, especially in liver and skeleton, and an increase of urinary excretion, often expressed as cumulative excretion (Nenot et al. 1971b, Stradling et al. 1993). There is however less consensus on the efficacy of DTPA on wound retention. Some authors reported that DTPA decreased wound-site activity, whereas others observed no change in wound-site retention (Volf 1974, Ilyin 2001). In the present study an effect of DTPA treatment at the wound site was not detected. The lack of agreement between studies could originate from two major sources of variation. Firstly, the experimental model of wound contamination may be critical regarding the access of Am to DTPA at the wound site, and subsequently on DTPA efficacy. Thus results obtained from simulated wound studies that used s.c., i.m. injection, or an incision for contaminant administration may not be directly comparable. Secondly, the determination of activity at wound site was particularly difficult as compared with systemic tissues. Instead of being well

localized, the contamination at wound site will be distributed in a 3-D manner, will vary from one animal to another, as well as with time after contamination. These factors are challenging for external measurements using detectors (HPGe, NaI) and may result in heterogeneous measurement geometries across animals and studies. The representation of wound retention for each rat in this work illustrated the interindividual variations that may be obtained from wound measurements, whereas experimental conditions were as controlled as possible. The large spread of the results indicated that wound monitoring carried out for workers should also be evaluated and interpreted with caution. In addition to wound measurements, it is crucial to follow-up the 24 h urinary Am excretion for worker monitoring after wound contamination. Despite the observed variability in wound counts, one day after contamination, there was 30%-50% of Am activity remaining at wound site for untreated rats, which is consistent with the strong retention category proposed by the NCRP (NCRP 2006). Data used by NCRP were obtained using i.m. contamination in rats mostly, whereas data from this paper were obtained for an actual wound contamination with breach of vascular integrity. In the future it would be useful to develop an approach that could "actively" remove Am that is bound to biological ligands at wound site particularly in the first 24 h after contamination.

The comparison of DTPA efficacy between times of single DTPA administration showed that the greatest increase in urinary Am excretion was achieved following DTPA administration on the day of wound contamination. In addition, tissue uptake of Am was rapid in untreated rats and this occured mainly within 24 h. As the human body has a slower metabolism than that of a rodent, it can be assumed that tissue uptake would be relatively slower. But immediate entry into the circulation, from a puncture wound for example, is an important factor and so the current recommandation still holds: DTPA should be administered to workers as fast as possible after wound contamination.

The efficacy of the local injection of DTPA on systemic tissue retention and urinary excretion was found similar as that of an i.v. injection at the same dose. A local injection of the hyperosmolar DTPA solution may be painful, the dose administered limited, and the administration possibly

difficult depending on the wound site, which may often be located on hands and extremities. In this case an i.v. injection may be preferred over a local injection to preserve a better comfort for the patient. Local rinsing of wounds using the DTPA solution should still be applied during primary care. In addition, wound dressings can be applied at the work place immediately after the incident during the transfer to the infirmary as described by Houpert and colleagues for U (Houpert et al. 2004).

This work showed that repeated i.v. injection of DTPA during the first days after contamination resulted in a moderate enhancement of Am excretion in urine as compared with a single i.v. injection at 30 min. These data confirmed that, despite the lasting effect of DTPA, it is valuable to repeat DTPA injections during the first few days after contamination. A chronic treatment in the long term may be necessary too depending on the particular case, but this consideration goes beyond the scope of the wound contamination experiments reported here. Recent results obtained in the laboratory after injection or inhalation of Pu provide indications that DTPA protocols need to be tailored for each patient and that DTPA inhalation is also a useful route of administration for decorporation therapy (Gremy et al. 2017, Miccoli et al. 2019).

CONCLUSION

This work evaluated and quantified the efficacy of several DTPA protocols after wound contamination of rats with Am nitrate. Different treatment parameters were addressed (route, time, frequency of administration) at several times after contamination. Statistical analyses carried out using STATBIODIS showed (i) specific urinary Am excretion profiles without and after treatment, (ii) rapid tissue uptake of Am that occurs within 24 h after contamination, (iii) a prompt and lasting effect of DTPA administered shortly after wound contamination, (iv) an equivalent efficacy of local and i.v. injection treatment, (v) relevance of early repeated injections. This work can contribute to a better interpretation of human cases of wound contamination and DTPA treatment.

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REFERENCES

Agence Nationale de Sécurité des Médicaments et des Produits de Santé. Piratome sheet #4: Ca-DTPA [online]. Available at: http://ansm.sante.fr/var/ansm_site/storage/original/application/51f7ff052c4d57aeef12bf9caf1ffeab.pdf. Accessed 03 April 2020.

Avtandilashvili M, Dumit S, Tolmachev SY. USTUR whole-body case 0212: 17-year follow-up of plutonium contaminated wound. Radiat Prot Dosim 178: 160-169; 2018.

Bates D, Machler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw 67: 48; 2015.

Bauer DF. Constructing confidence sets using rank statistics. Journal of the American Statistical Association 67: 687-690; 1972.

Bistline RW, Watters RL, Lebel JL. A study of translocation dynamics of plutonium and americium from simulated puncture wounds in beagle dogs. Health Phys 22: 829; 1972.

Boice JDJ. NCRP vision for the future and program area committee activities in 2017. Health Phys 114: 232-242; 2018.

Carbaugh EH, Lynch TP, Antonio CL, Medina-Del Valle F. Twenty-four years of follow-up for a Hanford plutonium wound case. Health Phys 99: 483-494; 2010.

Davesne E, Quesne B, De Vita A, Chojnacki E, Blanchardon E, Franck D. Optimization of routine monitoring of workers exposed to plutonium aerosols. Ann Occup Hyg 60: 969-76; 2016.

Dumit S, Avtandilashvili M, Strom DJ, McComish SL, Tabatadze G, Tolmachev SY. Improved modeling of plutonium-DTPA decorporation. Radiat Res; 2018.

Fritsch P, Sérandour AL, Grémy O, Phan G, Tsapis N, Abram MC, Renault D, Fattal E, Benech H, Deverre JR, Poncy JL. Simplified structure of a new model to describe urinary excretion of plutonium after systemic, liver or pulmonary contamination of rats associated with Ca-DTPA treatments. Radiat Res 171: 674-686; 2009.

Fritsch P, Serandour AL, Gremy O, Phan G, Tsapis N, Fattal E, Benech H, Deverre JR, Poncy JL. Structure of a single model to describe plutonium and americium decorporation by DTPA treatments. Health Phys 99: 553-9; 2010.

Grappin L, Berard P, Menetrier F, Carbone L, Courtay C, Castagnet X, Le Goff JP, Neron MO, Piechowski J. Treatment of actinide exposures: A review of Ca-DTPA injections inside cea-cogema plants. Radiat Prot Dosim 127: 435-9; 2007.

Gremy O, Coudert S, Renault D, Miccoli L. Decorporation approach after rat lung contamination with plutonium: Evaluation of the key parameters influencing the efficacy of a protracted chelation treatment. Radiat Res 188: 552-561; 2017.

Gremy O, Laurent D, Coudert S, Griffiths NM, Miccoli L. Decorporation of Pu/Am actinides by chelation therapy: New arguments in favor of an intracellular component of DTPA action. Radiat Res; 2016.

Griffiths NM, Coudert S, Renault D, Wilk JC, Van der Meeren A. Actinide handling after wound entry with local or systemic decorporation therapy in the rat. Int J Radiat Biol 90: 989-95; 2014a.

Griffiths NM, Coudert S, Wilk JC, Renault D, Angulo JF, Van der Meeren A. Combined drug and surgery treatment of plutonium-contaminated wounds: Indications obtained using a rodent model. Health Phys 106: 638-44; 2014b.

Griffiths NM, Van der Meeren A, Angulo JF, Vincent-Naulleau S. Research on the radiotoxicology of plutonium using animals: Consideration of the 3Rs-replace, reduce, refine. Health Phys 119: 133-140; 2020.

Griffiths NM, Wilk JC, Abram MC, Renault D, Chau Q, Helfer N, Guichet C, Van der Meeren A. Internal contamination by actinides after wounding: A robust rodent model for assessment of local and distant actinide retention. Health Phys 103: 187-94; 2012.

Houpert P, Chazel V, Paquet F. A local approach to reduce industrial uranium wound contamination in rats. Canadian Journal of Physiology and Pharmacology 82: 73-78; 2004.

Ilyin L. Skin wounds and burns contaminated by radioactive substances (metabolism, decontamination, tactics, and techniques of medical care). Medical management of radiation accidents, 2nd edition. Boca Raton, FL, USA: CRC Press; 2001; 363-419.

International Commission on Radiological Protection. The 2007 recommendations of the International Commission on Radiological Protection. ICRP Publication 103 Ann. ICRP 37(2-4); 2007.

International Commission on Radiological Protection. Occupational Intakes of Radionuclides: Part 1. ICRP Publication 130 Ann. ICRP 44(2); 2015.

International Commission on Radiological Protection. Occupational Intakes of Radionuclides: Part 4. ICRP Publication 141 Ann. ICRP 48(2/3); 2019.

Kastl M, Giussani A, Blanchardon E, Breustedt B, Fritsch P, Hoeschen C, Lopez MA. Developing a physiologically based approach for modeling plutonium decorporation therapy with DTPA. Int J Radiat Biol 90: 1062-1067; 2014.

Lamart S, Griffiths NM, Tchitchek N, Angulo JF, Van der Meeren A. Analysis methodology and development of a statistical tool for biodistribution data from internal contamination with actinides. J Radiol Prot 37: 296-308; 2017.

Lamart S, Van der Meeren A, Grémy O, Miccoli L, Coudert S, Dubois S, Bibard S, Serond AP, Angulo JF, Griffiths NM. Americium biodistribution in rats after wound contamination with different physicochemical forms in the presence or absence of plutonium: Analyses using STATBIODIS. J Radiol Prot 39: 707-738; 2019.

Mezaguer-Lekouaghet M, Souidi M, Badreddine A, Blanchardon E, Bertho JM, Benadjaoud MA, Baz A, Lounis-Mokrani Z. Biokinetics and dose assessment after iodine intake in a thyroidectomised rat model. J Radiol Prot 39: 292-308; 2019.

Miccoli L, Menetrier F, Laroche P, Gremy O. Chelation treatment by early inhalation of liquid aerosol DTPA for removing plutonium after rat lung contamination. Radiat Res 192: 630-639; 2019.

Miller G, Poudel D, Klumpp JA, Guilmette RA, Melo D. Second-order kinetics of DTPA and plutonium in rat plasma. Radiat Res 189: 64-67; 2018.

National Council on Radiation Protection and Measurements. Development of a biokinetic model for radionuclide-contaminated wounds and procedures for their assessment, dosimetry, and treatment: Recommendations of the National Council on Radiation Protection and Measurements. Bethesda, MD: NCRP; NCRP Report No. 156; 2006.

Nenot JC, Morin M, Lafuma J. Contaminations experimentales par l'americium en solution et leur traitement. Health Phys 20: 383-391; 1971a.

Nenot JC, Morin M, Lafuma J. Etude experimentale de la decontamination du squelette apres inhalation de nitrate d'americium. Health Phys 21: 395-400; 1971b.

Noguchi K, Gel YR, Brunner E, Konietschke F. nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments. J Stat Softw 50: 23; 2012.

Piechowski J, Cavadore D, Tourte J, Cauquil MH, Raynaud P, Harduin JC, Thomas P, Chaptinel Y. Model and practical information concerning the radiotoxicological assessment of a wound contaminated by plutonium. Radiat Prot Dosim 26: 265-270; 1989.

Piechowski J, Menoux B, Miele A, Grappin L, Guillermin A-M, Fottorino R, Ruffin M. Implications du médecin du travail et de l'expert dans la gestion et la dosimétrie d'un incident de contamination : Exemple

d'une plaie contaminée par le plutonium. Radioprotection 38: 29-50; 2003.

Poudel D, Bertelli L, Klumpp JA, Waters TL. Interpretation of urinary excretion data from plutonium wound cases treated with DTPA: Application of different models and approaches. Health Phys 113: 30-40; 2017.

Poudel D, Bertelli L, Klumpp JA, Waters TL. Some considerations for chelation treatment and surgical excision following incorporation of plutonium in wounds. Health Phys 114: 307-318; 2018.

R Core Team. R: A language and environment for statistical computing [online]. Available at: https://www.R-project.org/.

Stather JW, Smith H, Bailey MR, Birchall A, Bulman RA, Crawley FE. The retention of 14C-DTPA in human volunteers after inhalation or intravenous injection. Health Phys 44: 45-52; 1983.

Stevens W, Bruenger FW, Atherton DR, Buster DS, Howerton G. The retention and distribution of 241Am and 65Zn, given as DTPA chelates in rats and of [14C]DTPA in rats and beagles. Radiat Res 75: 397-409; 1978.

Stradling GN, Gray SA, Moody JC, Pearce MJ, Wilson I, Burgada R, Bailly T, Leroux Y, Raymond KN, Durbin PW. Comparative efficacies of 3,4,3-LIHOPO and DTPA for enhancing the excretion of plutonium and americium from the rat after simulated wound contamination as nitrates. Int J Radiat Biol 64: 133-140; 1993.

Sugarman SL, Findley WM, Toohey RE, Dainiak N. Rapid response, dose assessment, and clinical management of a plutonium-contaminated puncture wound. Health Phys 115: 57-64; 2018.

U.S. FDA. U.S. Food and drug administration FDA approved drugs to treat internal contamination from radioactive elements. FDA Press Release 4-78; 2004.

Volf V. Experimental background for prompt treatment with DTPA of 239Pu-contaminated wounds. Health Phys 27: 273-7; 1974.

LIST OF FIGURE CAPTIONS

Figure 1 : Individual 24 h urinary excretion of Am from day 1 to 7 after wound contamination with Am nitrate for untreated rats (left panel) and rats treated with a single intravenous injection of DTPA 2 h after contamination (right panel). Each colour represents one animal. The red arrow materializes the time of DTPA injection. Excretion functions are represented with continuous black lines. See Supplemental Figure 1 and Supplemental Figure 2 to visualize the comparison between the data and predictions for each animal for untreated and treated rats, respectively. Untreated rats were from experiments: AB_111, AB_151, AB_161, AB_162, AB_191 (N=18-32 data per time point). Treated rats were from: AB_151, AB_162 (N=5-11) depending on the time of euthanasia.

Figure 2 : Individual enhancement factor of 24 h urinary excretion of Am obtained with a single i.v. injection of DTPA 2 h after wound contamination. The horizontal black line represents an enhancement factor of 1.

Figure 3 : Individual Am activity retention in tissues and wound at one and seven days (top and bottom panels, respectively) after wound contamination with Am nitrate for untreated rats and rats treated with a single i.v. injection of DTPA 2 h after contamination (left and right panels, respectively) (% of Am administered activity). Each color represents the data from one animal. Untreated rats were from AB_151, AB_161, AB_191 at day 1 (N=14) and AB_111, AB_162 and AB_191 at D7 (N=18). Treated rats (i.v. injection at 2 h) were from AB_151 at day 1 (N=6) and AB_162 at D7 (N=5).

Figure 4 : Individual percentage of inhibition of Am retention in tissue from an i.v. injection of DTPA 2 h after wound contamination with Am nitrate.

Figure 5 : Individual Am activity retention ratios (Skeleton to liver, kidney to skeleton and kidney to liver) at one and seven days (top and bottom panels, respectively) after wound contamination for untreated rats and rats treated with a single i.v. injection of DTPA 2 h after contamination (left and right panels, respectively) (% of Am administered activity). Each color represents data derived from one animal.

Figure 6 : Individual 24 h urinary excretion of Am (% of Am administered activity) for seven days after wound contamination with Am nitrate and different treatment protocols with DTPA: i.v. or local injection (left and right panels, respectively) at 30 min, 2 h or 1 day after contamination. Arrows materialize these treatment times with colors matching the treatment group. Data surrounded by the box correspond to urine collected before DTPA administration for the groups of animals treated at day 1.

Figure 7 : Individual Am retention in tissue and wound (% of Am administered activity) at day 1 and 7 (top and bottom panels, respectively) after wound contamination with Am nitrate for different treatment protocols with DTPA: i.v. or local injection (left and right panels, respectively) at 30 min, 2 h or 1 day after contamination.

Figure 8 : Individual 24 h urinary excretions of Am from day 1 to 7 after wound contamination with Am nitrate for rats treated with a single i.v. injection of DTPA at 30 min (left panel) and with repeated i.v. injection of DTPA at 30 min, 1, 2 and 3 days (right panel). Each colour represents the data from one animal. Red arrows materialize the times of DTPA administration.

Figure 9 : Individual Am activity retention in tissues and wound seven days after wound contamination with Am nitrate for rats treated with a single i.v. injection of DTPA and rats treated with a repeated i.v. injection of DTPA at 30 min, 1, 2 and 3 days after contamination (left and right panels, respectively) (% of Am administered activity). Each color represents the data from one animal.

LIST OF TABLE CAPTIONS

Table 1 : Characteristics and DTPA treatment protocols of wound experiments carried out with Am nitrate in the laboratory and analyzed in this article. - : none for control groups.

Table 2 : Test of the overall effects of treatment, time after contamination and interaction between treatment and time on Am 24 h urinary excretion after wound contamination. A non-parametric test for independent (treatment effect) and paired (time effect) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Table 3 : Multiple comparisons of 24 h Am urinary excretion data (% of Am administered activity) for each time after wound contamination between untreated rats and rats treated with a single i.v. injection of DTPA at 2 h. The Mann–Whitney non-parametric test (or Wilcoxon rank sum test) for independent groups was used. The test evaluated whether data for treated rats were greater than those for untreated (unilateral alternative hypothesis, H1). CI: confidence interval.

Table 4 : Regression and correlation coefficients of the Am urinary excretion functions obtained from non-linear regression of the Am daily urine data after wound contamination with Am nitrate for untreated rats and rats treated with a single i.v. injection of DTPA 2 h after contamination; CI: confidence interval.

Table 5: Multiple comparisons of Am retention data for each tissue between untreated and treated rats one and seven days after wound contamination with Am nitrate. The Mann and Whitney non parametric test (or Wilcoxon rank sum test) for independent groups was used. The test evaluated whether data obtained with treatment were smaller than those without (unilateral alternative hypothesis H1). CI: confidence interval.

Table 6 : Multiple comparisons of individual 24 h urinary Am excretion data between a single i.v. injection DTPA injection and repeated DTPA i.v. injections after wound contamination with Am nitrate.

LIST OF SUPPLEMENTAL MATERIAL CAPTIONS

Supplemental Figure 1 : Individual 24 h urinary excretion of Am for seven days after wound contamination with Am nitrate for untreated rats. Comparison between experimental data (pink) and predicted values (blue) for each animal, codified as AB_XXX_XX.

Supplemental Figure 2 : Individual 24 h urinary excretion of Am for seven days after wound contamination with Am nitrate for rats treated with an intravenous injection of DTPA 2 h after contamination. Comparaison between experimental data (pink) and predicted values (blue) for each animal, codified as AB_XXX_XX.

Supplemental Figure 3 : Ratio of urinary excretion of Am between after and before DTPA administration, i.e., between day 2 and 1, for rats treated at day 1 by an intravenous injection.

Supplemental Table 1 : Summary statistics of retention data obtained for untreated groups and groups treated with a single i.v. injection of DTPA 2 h after wound contamination with Am nitrate. n: number of animals.

Supplemental Table 2: Test of the overall effects of treatment, tissue and interaction between tissue and treatment on Am tissue retention after wound contamination with Am nitrate for each time of euthanasia. A non-parametric test for independent (treatment effect) and paired (tissue effect) data was used, accounting for the animal as random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 3 : Test of the overall effects of the time after contamination, tissue and interaction between tissue and time on Am tissue retention after wound contamination for untreated and rats treated with a single i.v. injection of DTPA 2 h after contamination, respectively. A non-parametric test for independent (time of euthanasia effect) and paired (tissue effect) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 4 : Multiple comparisons of Am retention data for each tissue between the two times after wound contamination at day 1 and 7 for untreated and treated rats respectively. The Mann and Whitney non parametric test (or Wilcoxon rank sum test) for independent groups was used. The test evaluated whether data obtained at day 1 and 7 were different (bilateral alternative hypothesis H1). CI: confidence interval.

Supplemental Table 5 : Summary statistics for tissue ratio values obtained for untreated groups and groups treated with a single i.v. injection of DTPA 2 h after wound contamination with Am nitrate. n: number of animals.

Supplemental Table 6 : Test of the overall effects of treatment, tissue activity ratio and interaction between treatment and tissue ratio on Am tissue retention after wound contamination for each time after contamination. A non-parametric test for independent (treatment effect) and paired (tissue ratio effect) data was used, accounting for the animal as random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 7 : Multiple comparisons of Am tissue activity ratio data between untreated and treated rats one and seven days after wound contamination. The Mann and Whitney non parametric test (or Wilcoxon rank sum test) for independent groups was used. The test evaluated whether data obtained with treatment were smaller than those without (unilateral alternative hypothesis H1). CI: confidence interval.

Supplemental Table 8 : Test of the overall effects of treatment time, time after contamination and interaction between treatment time and collection time on Am urine excretion after wound contamination with Am nitrate for two treatment routes (intravenous and local). A non-parametric test for independent (treatment time effect) and paired (time after contamination) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 9: Multiple comparison of individual 24 h urinary excretion data of Am between treatment times of a unique intravenous or local injection. Comparisons were conducted from day 1 to 7 after wound contamination with Am nitrate.

Supplemental Table 10 : Summary statistics of retention data obtained depending on time and route of DTPA administration after wound contamination with Am nitrate. n: number of animals.

Supplemental Table 11 : Test of the overall effects of treatment time, tissue and interaction between treatment time and tissue on Am tissue retention after wound contamination with Am nitrate for two treatment routes (intravenous and local) and two times of euthanasia (day 1 and 7). A non-parametric test for independent (treatment time effect) and

paired (tissue) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 12: Multiple comparison of individual tissue retention data of Am between treatment times of an i.v. injection of DTPA. Comparisons were conducted separately at day 1 and 7 after wound contamination with Am nitrate.

Supplemental Table 13: Multiple comparison of individual tissue retention data of Am between treatment times of a local injection of DTPA. Comparisons were conducted separately at day 1 and 7 after wound contamination with Am nitrate.

Supplemental Table 14: Test of the overall effects of treatment route, time after contamination and interaction between treatment route and time on Am urine excretion after wound contamination with Am nitrate for three treatment times (30 min, 2 h and 1 day). A non-parametric test for independent (treatment route effect) and paired (time of urine collection) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 15 : Multiple comparisons of individual 24 h urinary excretion data of Am between a local and an i.v. injection of DTPA for each treatment time (30 min, 2 h and 1 day). Comparisons were conducted from day 1 to 7 after wound contamination with Am nitrate.

Supplemental Table 16 : Test of the overall effects of treatment route, tissue and interaction between treatment route and tissue on Am tissue retention after wound contamination with Am nitrate for three treatment times (30 min, 2 h and 1 day). A non-parametric test for independent (treatment route effect) and paired (tissue) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 17 : Multiple comparisons of individual tissue retention data of Am between a local and an i.v. injection of DTPA for treatment times at 2 h and 1 day. Comparisons were conducted separately at day 1 and 7 after wound contamination with Am nitrate.

Supplemental Table 18 : Test of the overall effects of treatment frequency, time after contamination and interaction between treatment frequency and time on Am urine excretion after wound contamination with Am nitrate. A non-parametric test for independent (treatment frequency effect) and paired (time) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 19 : Summary statistics of retention data obtained depending on frequency of DTPA administration after wound contamination with Am nitrate. n: number of animals.

Supplemental Table 20 : Test of the overall effects of treatment mode, tissue and interaction between treatment frequency and tissue on Am tissue retention after wound contamination with Am nitrate. A non-parametric test for independent (treatment frequency effect) and paired (tissue) data was used, accounting for the animal as a random effect (f1.LD.f1 function from the {nparLD} R package).

Supplemental Table 21 : Multiple comparisons of individual tissue retention data of Am between a single i.v. injection of DTPA and a repeated i.v. injection at 30 min, 1, 2 and 3 days.