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In vitro assessment of plutonium uptake and release using the human macrophage-like THP-1 cells

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ABSTRACT

Plutonium (Pu) intake by inhalation is one of the major potential consequences following an accident in the nuclear industry or after improvised nuclear device explosion. Macrophages are essential players in retention and clearance of inhaled compounds. However, the extent to which these phagocytic cells are involved in these processes highly depends on the solubility properties of the Pu deposited in the lungs. Our objectives were to develop an *in vitro* model representative of the human pulmonary macrophage capacity to internalize and release Pu compounds in presence or not of the chelating drug diethylenetriaminepentaacetate (DTPA).

The monocyte cell line THP-1 was used after differentiation into macrophage-like cells. We assessed the cellular uptake of various forms of Pu which differ in their solubility, as well as the release of the internalized Pu. Results obtained with differentiated THP-1 cells are in good agreement with data from rat alveolar macrophages and fit well with *in vivo* data. In both cell types, Pu uptake and release depend upon Pu solubility and in all cases DTPA increases Pu release.

The proposed model may provide a good complement to *in vivo* animal experiments and could be used in a first assessment to predict the fraction of Pu that could be potentially trapped, as well as the fraction available to chelating drugs.

1. Introduction

Plutonium (Pu) compounds are widely used in the nuclear industries, and accidental exposure of workers may occur during nuclear fuel reprocessing. In addition, the release of actinides, such as Pu in the environment after a nuclear accident or the potential use of a radiological dispersal device, represents a public threat. For all of these scenarios, inhalation is one of the most likely routes of contamination, and may concern various physico-chemical forms of Pu such as oxides and nitrate. Pu distribution among organs and tissues depends on its physico-chemical form and the route of administration (ICRP, 1986; Fouillit et al., 2004; Weber et al., 2014). Once in the systemic compartment, Pu will deposit mainly in liver and skeleton. Understanding the fate of inhaled actinides compounds and the mechanisms that control actinide retention and clearance from the lungs are essential to evaluate the risk of long term deleterious effect and to design better targeted decorporation strategies.

The ICRP adopted the human respiratory tract model to calculate the radiation dose following intake of airborne radionuclides. This model classifies materials according to their absorption into blood in three types: Fast (F), Moderate (M) or Slow (S). Actinide oxide compounds (type S) undergo slow clearance from the lungs. Pu nitrate belongs to the type S or M of materials (ICRP 66, 1994). However, during the initial clearance phase which is completed within days, a fraction of the initially deposited material may be transformed into poorly soluble polymeric Pu forming colloids. These particles may be engulfed and stored into macrophages or scar tissue. Several groups have shown that the long term retention of Pu nitrate in the lungs participates to the inflammatory response (Van der Meeren et al., 2012; Nielsen et al., 2014).

The data used to build ICRP respiratory tract model are from animal experiments (dogs, rats, mice, non-human primates), as well as from human contamination cases. Some data from *in vitro* dissolution experiments were also considered. For most of these experiments, alveolar macrophages were collected by broncho-alveolar lavages from animals contaminated mainly with actinide oxides. A fair estimation of solubility properties of the inhaled compound was obtained using this model (Kreyling, 1992). However, *in vitro* models have been used mostly to describe the solubility of inhaled particles, but did not explore mechanisms of uptake or release, nor aimed at determining the portion of internalized compound accessible to chelating drugs.

When studying long-term clearance of actinides from the lung, assessment of macrophage retention is critical. Following inhalation of

Abbreviations: Pu, plutonium; DTPA, diethylenetriaminepentaacetate.

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insoluble compounds, including actinide oxides, macrophages are the first line of defence, and are key players in lung retention. Both mechanical clearance by mucociliary action and translocation of deposited particles in the respiratory tract by particle dissolution are considered as important clearance mechanisms. The relative importance of these two clearance pathways varies with physico-chemical properties of the inhaled compounds. Moreover, the ability of macrophages to engulf inhaled compounds depends upon various parameters, among which are particle size and shape. In addition to the role of macrophages on actinide retention, these cells also influence the dose delivered to the surrounding ones, such as epithelial cells. Indeed, homogeneous deposition of Pu in extracellular fluids or retention in macrophages will determine the cell population exposed to ionizing radiation, due to the short range of α rays (<100 μm in tissues). After phagocytosis, intracellular dissolution may occur. The solubility properties of the inhaled Pu compound determine the translocation rate from the lungs and subsequent transfer of dissolved material to blood.

Calcium diethylenetriaminepentaacetate (Ca-DTPA) is the only approved decorporation agent for Pu. It is highly efficient in decorporating soluble Pu from blood but remains barely efficient on poorly soluble material as well as on intracellular actinides. Therefore, a critical step to improve chelating drug efficacy is the understanding of intracellular chelation of Pu, which is usually considered low or even not occurring.

In order to further understand the biological fate of inhaled actinides, we initiated and characterized an *in vitro* macrophage-like model to: i) predict *in vivo* behavior of actinides after pulmonary contamination and ii) better describe the accessibility of Pu to chelating agents to predict and improve local efficacy of decorporating drugs. THP-1 cells and are widely used for toxicology studies after differentiation into macrophage-like cells. The present study reports firstly the characterization of their differentiation into human macrophage-like THP-1 cells under our experimental conditions. Secondly, we evaluated the uptake of various physico-chemical forms of Pu and the release of Pu from these cells, in the presence or not of DTPA. The use of three chemical forms of Pu allows the comparison of preformed Pu colloids, Pu potentially able to form colloids at physiological pH (Pu nitrate), and stable monomeric soluble forms (Pu citrate). To ensure the validity of our model, data were compared to those obtained using rat alveolar macrophages, representing fully differentiated cells. Our results are then compared to previously published *in vivo* data.

The present model will make possible the investigation of some of the basic mechanisms of actinide uptake and release. It may also provide a tool that can be used to screen potential chelating drugs, and optimize the solubilization of intracellular actinide particles.

2. Materials and methods

2.1. Plutonium compounds

Three different physico-chemical forms of plutonium were prepared and will be referred as citrate, nitrate or colloids. They were prepared from a stock solution of Pu (12.5% in activity ^{239}Pu and 86% ^{238}Pu, CEA, France) in 2 N HNO_3 as previously described (Van der Meeren et al., 2012). Briefly, following evaporation of stock solutions, the “citrate” and “nitrate” compounds, were made so final concentrations were 20 mM citrate and 25 mM nitrate respectively. For the “colloid” compound, colloids were formed *in vitro* as follows: after evaporation of the stock solution, Pu was diluted so Pu concentration was 25 μM and nitrate concentration was 25 mM then the pH was elevated at 11 with NaOH.

The physical state of these solutions was determined by filtration through a 10 nm filter (VFWP 025, Millipore) placed in a filtration device. The filter was then rinsed twice with water.

2.2. Cell culture

2.2.1. Broncho-alveolar lavages and cell culture

Male Sprague Dawley rats were obtained from Charles River Laboratories (l'Arbresle, France), housed 5 or 6 to a cage and maintained at constant temperature on a 12:12 h light-dark cycle. They received commercial rodent chow and water *ad libitum*. Housing and experiments were carried out in compliance with the French regulation for animal experimentation (European act 86/609/EEC, November 24, 1986). After anesthesia with Nembutal (sodium pentobarbitone, 40 mg/kg, Cerva Santé Animal, Libourne, France), rats were perfused in the left ventricle with Phosphate Buffered Saline (PBS) to remove most of the blood from pulmonary circulation. Then, after cannulation of the trachea, broncho-alveolar lavages (BAL) were carried out with warmed sterile PBS (Phosphate Buffered Saline, Life Technologies). The total number of nucleated cells in BAL was determined. After centrifugation of BAL, over 95% of recovered cells were alveolar macrophages (AMs). Cells were resuspended in complete RPMI medium containing 200 mM L-Glutamine (Sigma Aldrich, France) and supplemented with 10% FBS (Fetal Bovine Serum, Techgen, France).

The THP-1 cell line (ATCC, France) is derived from the blood of a boy with acute monocytic leukemia (Tsuchiya et al., 1980). THP-1 cells were routinely maintained in complete RPMI medium containing 10% FBS, at a seeding concentration of $2–3 \cdot 10^5$ cells/ml. Prior to contamination with Pu, the THP-1 cells in suspension were used non differentiated or differentiated into a macrophage-like cell by adding 50 nM PMA (phorbol 12-myristate 13-acetate, Sigma-Aldrich) or 50 nM vitamin D3 (vitD3, Cholecalciferol (D3), Sigma-Aldrich) for 3–4 days. Adherent cells were then detached with trypsin-EDTA 0.25% (Life Technologies, France) and resuspended in complete medium.

2.3. Pu uptake and dissolution

For Pu uptake studies, cells were initially incubated with the different physico-chemical forms of Pu (10 kBq/10^6 cells, corresponding to 0.56 μg Pu/10^6 cells) in 2 ml of medium in conical plastic tubes to maintain cells in a non-adherent state. After 2 h incubation at 37 °C, cells were washed twice with PBS to remove non internalized Pu, and counted. The preliminary phagocytosis step was used to avoid the presence of free Pu in the insert wells. Total α activity associated with cells was determined by liquid scintillation counting (Tricarb Packard counter) in Ultimagold (Packard, France). Pu uptake (%) is expressed as the fraction of Pu associated with cells divided by the initial activity added to the cells (in Bq/cell) × 100. In addition these cells were cytocentrifuged onto microscope slides and processed for autoradiography. For this purpose, slides were covered with photographic emulsion (NTB 2K-5, Kodak, Healthcare, USA) and exposed for 7 days. Slides were then counterstained with hemalun.

For release studies, 10^5 Pu-loaded cells (AMs or THP-1) were placed in 24 well culture inserts (Transwell®, Costar, pore size of 0.4 μm). Sampling of medium in the receiver well was done at 2 h, then at days 1, 2, 3 and 7 after plating. At each collection time, the full volume of medium in the receiver well was collected and replaced by an equivalent volume of fresh medium (700 μl). Samples were then returned to 37 °C incubation for the indicated periods of time (*e.g.*, 2 h to 7 days). At Day 7, the insert content (medium + cells) was collected in addition to the medium from receiver well as for the other time points. Cells were lysed with triton 0.5% and added to the collected insert medium in a scintillation vial for counting. The total activity recovered over 7 days is then compared to the initial activity added in the insert well. The percentage of release is expressed as cumulated activity measured in the lower compartment at each sampling time/initial activity in insert well × 100.

The consequences of DTPA treatment on Pu transfer were assessed as follows: At the initiation of the experiment, DTPA 0.5 mM (Pharmacie