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NOVEL DRUG DELIVERY SYSTEMS FOR ACTINIDES (URANIUM AND PLUTONIUM) DECONTAMINATION AGENTS

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Abstract

The possibility of accidents in the nuclear industry or of nuclear terrorist attacks makes the development of new decontamination strategies crucial. Among radionuclides, actinides such as uranium and plutonium and their different isotopes are considered as the most dangerous contaminants, plutonium displaying mostly a radiological toxicity whereas uranium exhibits mainly a chemical toxicity. Contamination occurs through ingestion, skin or lung exposure with subsequent absorption and distribution of the radionuclides to different tissues where they induce damaging effects. Different chelating agents have been synthesized but their efficacy is limited by their low tissular specificity and high toxicity. For these reasons, several groups have developed smart delivery systems to increase the local concentration of the chelating agent or to improve its biodistribution. The aim of this review is to highlight these strategies.

Keywords: Actinides – Chelating agents – Decontamination – Decorporation – Lung delivery – Liposomes – Nanoemulsions

1. Introduction

The safety of the nuclear industry is generally considered good, but accidents do still happen worldwide. The threat of a nuclear terrorist event involving the release of radionuclides should also be considered. Accidental exposure to radionuclides can occur by inhalation or ingestion or via wounds during industrial application, waste disposal, and warfare {Zamora, 1998 #947}. Among actinides, highly active isotopes of plutonium (Pu), americium (Am) and to a lesser extent uranium (U), are considered as dangerous. In this review, we focused on U and Pu mainly, because of their widespread use in the nuclear industry which has led to many studies in drug design and delivery. Nevertheless, one should not forget the danger of other radionuclides such as Strontium-90, Iodine-131, Cesium-134, Cesium-137, Ruthenium-103 or Ruthenium-106 which are the other principal harmful radionuclides following a nuclear reactor accident.

Natural U is composed of three isotopes existing in different proportions in mass: U-234 (0.0054%), U-235 (0.7110%) and U-238 (99.2836%). The two most abundant isotopes on earth are U-238 and U-235. U-234 is produced by α decay of U-238 (emission of a helium nucleus) and represents a small part of the total mass of U. However, it is the most radioactive isotope of U. U-235 is the only natural and readily fissile isotope which releases energy under neutrons particles bombardment. This nuclear property explains the use of the U-235 isotope for energy production in nuclear reactors. Different forms of enriched, depleted or reprocessed U exist depending on the proportions of the three isotopes mentioned. For example, enriched U comprises 3 to 5% U-235 for civilian applications, and more than 90% for military applications. This highly enriched U might induce radiological toxicity. Conversely, depleted U comprises a lower amount of this isotope than natural U, and is consequently less radioactive. The major industrial applications of enriched U are the production of energy in nuclear power plants while depleted U is mainly used for the manufacture of armor and ammunition. Owing to the low specific activity and long half-life of its main radioisotope U-238, natural or depleted U is not considered to be a radiological hazard but can induce a non-negligible chemical toxicity.

Pu is almost entirely of artificial origin but can be found in trace amounts in U ores (Pu-239) or rare earth (Pu-244). The 15 isotopes of Pu from Pu-232 to Pu-246 are all radioactive. The most frequently encountered in the nuclear industry are the isotopes Pu-238, Pu-239, Pu-240 and Pu-241. The different isotopes of Pu are produced from U in nuclear reactors. Two main industrial applications are developed from the isotopes Pu-238 and Pu-239. Pu-238 is used as a source of thermoelectric energy and is a component of pacemaker batteries, satellites and spacecraft. Pu-239 is mainly used as fissile material in some nuclear power reactors for electricity production. In France and in Europe, it is used along with U as a mixture of oxides (MOX) resulting from processing operations of spent U fuel. In pessimistic scenarios, civilians could be exposed to radioactive aerosols in case of nuclear accidents.

The main exposure routes to U and Pu are oral, lungs and intact or injured skin. In all cases, the radionuclides can reach the blood and damage several tissues. Although many chelating agents have been synthesized in the recent years, little is known about their biopharmaceutical properties and toxicity. Well-known old chelating agents are poised with a low tissue specificity together with a high toxicity, slowing down their clinical development. For these reasons, most of these active molecules have been reformulated in the light of the progress achieved in the field of local and systemic delivery to increase their efficacy and reduce their toxicity. These approaches will be discussed in the present review.

2. Exposure to uranium and plutonium and consequences

2.1. Modes of exposure

Exposure to radionuclides can occur either by an external exposure when the radionuclide remains outside or at the surface of the body, or by an internal exposure when the radionuclide is incorporated or absorbed into the body either by inhalation, ingestion, through intact skin or through wounds. Following external exposure, the radionuclide can induce tissue irradiation which can be damaging depending on the nature of the emitted radiation. The risk is higher if the radiation is penetrating, which is the case of gamma or X radiations, and to a lesser extent, beta radiation. The risk of external exposure can be easily lowered or minimized by shielding the individual or by removing the radiation source. Following internal exposure, the radionuclide is incorporated into the body and the damage

induced by irradiation of cells or tissues at the vicinity of the element can be of concern, whatever the radiation type, and especially in the case of highly ionizing alpha particles which transfer their energy more rapidly and at shorter range than gamma radiation for instance. Hence, since the major isotopes of the actinides U and Pu are mostly alpha emitters, the toxicological consequences related to the incorporation of these elements can be quite concerning. This mode of exposure to both actinides mainly depends on the industrial or military uses of the different compounds.

Inhalation is considered as the most frequent mode of contamination in the industry. It can occur after an explosion or a fire, causing atmospheric dispersion of radionuclides in case of containment disruptions. The second most frequent mode of contamination after inhalation is skin exposure. This can occur especially on injured skin, after an explosion or improper handling of contaminated tools or sharps inside a glove-box. The skin can also be contaminated by contact with aerosols or by contact with surfaces contaminated with radionuclides. Ingestion is unlikely to be a frequent mode of contamination among workers in the nuclear industry because it is minimized by health and safety instructions. However, it may be more critical for civilians in the case of an accidental release of radioactivity into the environment {Gerber, 1992 #463} occurring after an accident such as the one that took place in Chernobyl or more recently in the Fukushima Dai-ichi Nuclear Power Plant {Schneider, 2013 #1261;Butler, 2011 #1265;Sarin, 2011 #1266}.

For illustration purpose, the frequencies of contamination that can occur in French nuclear plants or research centers were published in two different reports in 2004 and 2007 {Blanchin, 2004 #394;Grappin, 2007 #1267}. The first report on the management of individuals potentially contaminated after internal exposure during incidents occurring between 1996 and 2002, shows that 88% of the 1,529 incidents treated during this period were recorded as suspected inhalation and 11% as suspected contamination through wounds {Blanchin, 2004 #394}. The second report in which 548 cases of exposure to actinides were recorded between 1970 and 2003, shows that dermal contamination in the presence or absence of injury is significant since it is the first route of contamination, with 53.8% of cases, followed closely by inhalation with 39.5% of cases {Grappin, 2007 #1267} (Figure 1).

2.2. Biodistribution

As shown in Figure 2, whatever the exposure route, the actinides will follow a particular pathway that will affect several particular tissues. This section deals with the biodistribution of two actinides : U and Pu.

2.2.1. Uranium

Occupational activities involving the handling of U in different forms entail possible contamination of exposed workers. To better predict and prevent the toxic effects of U, it is necessary to understand the mechanisms of its absorption. The absorption of U depends on its physicochemical form, the solubility of the compounds and the mode of contamination. The more soluble forms such as uranyl nitrate $\text{UO}_2(\text{NO}_3)_2$ or ammonium uranyl tricarbonate $(\text{NH}_4)_4\text{UO}_2(\text{CO}_3)_3$ are more diffusible. Ammonium diuranate $(\text{NH}_4)_2\text{U}_2\text{O}_7$ or uranyl acetate $\text{UO}_2(\text{CH}_3\text{COO})_2$ are less soluble and therefore less diffusible. Finally, U dioxide UO_2 is less soluble {De Rey, 1983 #410}. Soluble forms, such as nitrate, can release uranyl ions which will be distributed to target organs such as the kidneys and bones. The least soluble forms have the tendency to be retained in organs and then to express their toxicity locally.

The gastrointestinal absorption of U in hamsters is about 0.8% for uranyl nitrate and 0.11% for U dioxide {Harrison, 1991 #797}. In humans, this value is estimated to vary from 1.3 to 1.9% according to the different studies available in the literature {Wrenn, 1985 #1271}. The International Commission on Radiological Protection (ICRP) considers this value to be 2% {Harrison, 1991 #797}, except for relatively poorly soluble tetravalent compounds such as UO_2 , U_3O_8 and UF_4 for which the digestive absorption is only 0.2% {International Commission on Radiological Protection, 1997 #1325}.

As far as the respiratory pathway is concerned, military activities can generate U aerosols in case of explosion of shields or ammunition made of depleted U. These aerosols can also be found in industrial activities involving U. The main route of absorption of these particles is the pulmonary tract. In rats, it was shown that 26.2% of the amount inhaled nasally is found in the lungs, more particularly in the deepest areas. The ICRP defines three types of compounds according to their solubility in lungs: type F (very transferable) for compounds

with a half-life of absorption of 10 minutes, type M (moderately transferable) for compounds with a half-life of 140 days, and type S (slowly transferable) for compounds with a half-life of more than 7,000 days. The absorption is approximately 2% for type F and M compounds, and 0.2% for type S compounds {International Commission on Radiological Protection, 1997 #1325}. The solubility not only depends on the chemical form of U, but also on the specific surface area of U particles {Chazel, 1998 #1391} and on particle size {Chazel, 2003 #1284}. For instance, slowly transferable particles are removed by the mucociliary escalator and eventually transported to the mouth and swallowed, ending up in the gastrointestinal tract {International Commission on Radiological Protection, 1994 #794}.

The incorporation and distribution of actinides after its translocation through the skin or a wound is described by the biokinetic model for radionuclide-contaminated wounds developed by the US National Council on Radiation Protection and Measurements (NCRP), in collaboration with ICRP {National Council on Radiation Protection and Measurements, 2006 #783}. This multicompartment model uses first-order linear biokinetics to describe the retention and clearance of a radionuclide deposited in a wound site. U deposited on intact or wounded skin is able to cross the tissue to a larger extent in case of wounds {Grives, #1393; Spagnul, 2011 #661}. Experiments performed *ex vivo* using Franz cells shows that U deposited in the form of uranyl nitrate, containing U-233, diffuses through rat skin in 3 hours and through pig ear skin in 4 hours {Petitot, 2004 #181}. It is noteworthy that rat skin is more permeable than pig skin. Pig skin, on the other hand, is closest in terms of permeability to human skin {Jung, 2015 #1387}. The amount of U diffusing across the skin after 24 hours corresponds to around 2.7% of the amount deposited in rats and around 42% in pigs. In the case of excoriated skin, that is to say skin where the cornea layer is removed, U can diffuse through in less than 30 minutes, in greater proportion in rats (78%) and in proportions of the same order of magnitude as found in intact skin in pigs (64%) {Petitot, 2004 #181}. Furthermore, *in vivo* experiments, conducted on depleted U, in rats show that U can be found in the dermis 30 minutes after contamination with a uranyl nitrate solution, and, after 6 hours, in muscles and kidneys {Petitot, 2007 #426}. Uranyl nitrate in powder form can also diffuse very rapidly through rat skin and ends up in the dermis of intact skin as rapidly as 30 minutes after contamination, and can be found in urine in less than 2 hours when the uranyl

nitrate powder is deposited on skin after excoriation {Petitot, 2007 #484}. This quick passage of the first skin barriers including the horny layer or *stratum corneum*, implies the need for an emergency decontamination performed immediately after contact with contaminated solutions. Alterations of the *stratum corneum* by burns increase the diffusion of U {Petitot, 2007 #484;Petitot, 2010 #538}.

Besides, in the context of military activities, fragments of depleted U can be generated and spread during explosions of ammunition or armor. These fragments can be embedded in the body and particularly in muscles. Medical procedures recommend to avoid their removal by surgery in order to prevent further release of U and subsequent redistribution to other tissues, mainly the kidneys and the bones, but also to other organs such as the liver, the spleen and the brain {McClain, 2001 #799;Pellmar, 1999 #488}.

Once absorbed, U under the form of $^{233}\text{UO}_2$ (particles less than 4 nm) and ^{233}U -uranyl nitrate is found in the blood and then complexed to three types of compounds {Cooper, 1982 #1327}: i) Low molecular weight ions, such as citrate or bicarbonate to about 60%, ii) Proteins, mainly transferrin, which accounts for about 50% of the proteinic ligands of U {Ansoborlo, 2006 #721}, although these complexes are less stable than those formed with citrates and bicarbonates, and iii) Lipoproteins. However, recent studies based on proteomics have investigated more deeply the association of U to serum proteins. It was evidenced that U was preferentially associated to proteins such as fetuin {Basset, 2013 #1362} or osteopontin {Qi, 2014 #1361}. Since these proteins play a major role in bone formation, the strong distribution of U in the skeleton might arise from this association.

Elimination of U is mainly achieved by urinary excretion which accounts for 60 to 86% of the absorbed dose. Fecal elimination of U accounts for only 1 to 2%. In addition, the total U excretion rate is quite fast since 60 to 70% of the dose is excreted in the first 24 hours. Binding to target organs is also very fast and can occur in less than 30 minutes after the U has reached the general circulation {Wrenn, 1994 #1328}. About 20% of the plasma quantity of U is found bound to the proximal tubular cells of the kidneys. This binding increases when the urine pH decreases {Wrenn, 1985 #1271}. U can accumulate in some parts of the brain. In rats, chronic administration of uranyl nitrate via drinking water causes the accumulation

of U in the striatum, hippocampus and frontal cortex {Bussy, 2006 #802;Lestaevel, 2005 #801}.

2.2.2. Plutonium

The possible pathways of Pu incorporation into humans are primarily ingestion and inhalation. In addition, in nuclear industry, internal contamination of workers can also result from injuries or transcutaneous passage depending on the chemical nature of the compounds manipulated.

Pu is considered poorly transferable after ingestion. Each Pu compound is characterized by a factor of gastrointestinal absorption f_1 which values are between $1 \cdot 10^{-5}$ (less transferable compounds Type S, such as insoluble oxides) and $5 \cdot 10^{-4}$ (moderately transferable compounds Type M, such as nitrates) in publications 72 and 78 of the ICRP {International Commission on Radiological Protection, 1996 #1324;International Commission on Radiological Protection, 1997 #1325}. It should be noted that the intestinal absorption coefficient f_1 is subsequently recalculated and replaced by the alimentary tract transfer factor f_A which takes into account the new model of human alimentary tract proposed by the ICRP publication 100 {International Commission on Radiological Protection, 2006 #795}.

Inhalation of Pu particles can be a significant source of incorporation and irradiation of the lung which is a primary retention organ. Indeed, according to ICRP {International Commission on Radiological Protection, 1994 #794}, the transfer of Pu from lung to the blood depends on the particle diameter, the specific surface area and the solubility of the compounds defined by the coefficients of absorption to the blood. The compounds are classified as Type F (very transferable), type M (moderately transferable) or type S (slowly transferable). For example, insoluble Pu oxide particles are slowly solubilized into the lungs from where they are removed with a biological half-life of about 500 to 1, 000 days. As a matter of fact, early animal studies have demonstrated differences in the biokinetics of inhaled oxides of Pu-238 and Pu-239. Pu-238 exhibits a substantially more rapid translocation from the lungs to the systemic organs, particularly the skeleton. This resulted in the predominant occurrence of skeletal cancers in animals exposed to Pu-238 oxides while lung cancer occurrence was more important in animals exposed to Pu-239 oxides {Suslova,

2012 #1360}. Elimination is then achieved either by the fecal pathway after clearance of lung macrophages or inhaled particles, or by particle transfer and retention to the thoracic ganglia {International Commission on Radiological Protection, 1994 #794}.

Regarding skin contamination, Pu biokinetics can be described by the same multicompartment model described above for U. A recent study was conducted in rats contaminated with Pu-oxides or Pu-nitrate following wounding by deep incision of the hind leg {Griffiths, 2012 #701}. The authors demonstrated that, although the activity transferred from wound to blood was higher after contamination with a moderately soluble form of Pu-nitrate than Pu-oxides, 7 days after contamination most of the MOX or Pu-nitrate was retained at the wound site conversely to U. Rapid actinide retention in liver and bone was observed within 24 hours, which increased up to 3 months. After 3 months, around 95% of the activity remained at the wound site, and excretion of the actinides was extremely low {Griffiths, 2012 #701}.

Regarding the whole tissue distribution, recently, Weber et al {Weber, 2014 #1395} have examined the body distribution of Pu-239 in rats following a single administration by either intravenous (IV) injection, inhalation or by intramuscular (IM) administration to mimic a wound. They have demonstrated that by 1 hour post exposure, the skeleton contained the largest fraction of Pu-239, mainly after IV administration (50%). Skeleton was also the major Pu compartment after wound (40%), 6 days following exposure. For inhalation 0.4% of the activity was observed in the skeleton at early time-points before slowly increasing to 1% after 28 days. The second retention organ in terms of activity was found to be the liver. Following IV administration, 20% of activity was observed 2 days after contamination and for IM, 6% after 4 days. Following inhalation exposure, 0.5% of activity was found in liver 28 days after contamination. A more recent study with Pu-239 was conducted in rats and dogs {Melo, 2014 #1396} and concluded on a larger uptake by dog liver than rats speculating that the canine model would be the best to evaluate the efficacy of chelating agents {Melo, 2014 #1396}. Regarding the influence of the chemical nature of Pu, Fouillit et al. {Fouillit, 2004 #1392} showed that once in the blood after IV administration, Pu citrate and Pu nitrate were predominantly retained in the skeleton within the first hours after injection, whereas most of the Pu was in the liver after injection of Pu phytate {Fouillit, 2004 #1392}.

Once in blood, Pu is transported mostly bound to the extracellular iron transport protein transferrin and the intracellular iron storage protein ferritin {Boocock, 1970 #1365;Boocock, 1965 #1366}. However the specific molecular pathways of Pu entrance and traffic in cells was not understood until recently. Using PC12 as a cellular model, Aryal et al. {Aryal, 2012 #1364} showed that in addition to the iron binding proteins transferrin and ferritin, which have long been known to bind Pu, other proteins interact with Pu and could be involved in intracellular Pu trafficking and sequestration in mammalian cells. From blood, more than 80% of Pu is quickly transferred to the target organs and a residual fraction of less than 10% is excreted in the urine. Bones and liver then represent the two main retention organs in which approximately 50% and 30%, respectively, of the blood load are found in adults. A small fraction is then eliminated by the bile.

The biological half-life of Pu elimination is around 50 years in the bones and about 20 years in the liver. Finally, bone remodeling contributes to release of a soluble portion of Pu to the blood. The effective period of elimination of a radionuclide deposited in the body or in a particular tissue, is defined as the combination of its biological elimination period (which depends on the metabolic processes of the body) and its physical decay period (which depends on the nuclear characteristics of the radioactive element) (equation 1):

$$T_{eff} = \frac{T_{bio} \times T_{phys}}{T_{bio} + T_{phys}} \quad (1)$$

Where T_{eff} is the Effective elimination period; T_{bio} is the Biological elimination period and T_{phys} is the Physical elimination period.

According to ICRP publication 30, the effective period of elimination of the Pu-239 isotope may be linked to its biological half-life given its long physical half-life (24390 years) {International Commission on Radiological Protection, 1988 #1323}. The effective period of elimination of other isotopes is usually shorter than their biological elimination period ($T_{eff} < T_{bio}, T_{phys}$). Experimental data obtained in animals show that the distribution and retention of Pu in the liver and bones may vary depending on the physicochemical form of the contaminant injected into the bloodstream (citrate, nitrate, insoluble particle of Pu, etc.) but also as a function of the age and the species studied {International Commission on

Radiological Protection, 1986 #793}. For instance, in general, Pu polymeric forms are more retained in the liver than soluble monomeric forms, and bone deposit levels are higher in young growing animals.

2.3. Toxicological consequences

2.3.1. Uranium

U presents a dual toxicity, first as a chemical since it is a toxic heavy metal, second as radiological toxicity depending on the type of isotope and the degree of U-235 enrichment of the compounds. Therefore, in the case of contamination with enriched U, the radiochemical risk will be more significant the higher the activity of the U compound. Conversely, in the case of contamination with natural or depleted U, the toxicity is mainly due to its chemical properties {Leggett, 1989 #1337;Diamond, 1998 #1351;Souidi, 2009 #803}.

As far as the chemical toxicity of U is concerned, a review of data from the literature showed that death produced by large acute doses of soluble U was observed in experimental animal models with variations in the sensitivity among species {Kathren, 2008 #609}. The chemical toxicity of U in the kidneys is one of the consequences of the precipitation and binding of the radionuclide to the proximal tubular cells, the threshold dose being about $3 \mu\text{g}\cdot\text{g}^{-1}$ of kidney {Wrenn, 1985 #1271}. Indeed, injection of uranyl nitrate in rats leads to a decrease in the glomerular filtration rate. As a consequence, it can damage the renal tubules and reduce glomerular permeability {Blantz, 1975 #804}. These two mechanisms lead to acute renal failure. The renal toxicity is also found after transdermal passage of U {Lopez, 2000 #224}. The lethal dose LD_{50} through this route of exposure in the rat is about $2 \text{mg}\cdot\text{kg}^{-1}$ {Diamond, 1998 #1351}.

The skeleton is a long-term retention organ of U where the radionuclide replaces the calcium in the hydroxyapatite structure {Priest, 1983 #1329}. This accumulation causes a decrease in osteogenesis and therefore of bone mass. This effect is found both in the context of chronic {Diaz Sylvester, 2002 #805} and acute poisoning {Guglielmotti, 1989 #806;Ubios, 1991 #807}. Inhibition of osteogenesis by U is due to ultrastructural damage produced by the oxidative

stress on osteoblasts which are the cells responsible for the bone synthesis {Tasat, 2007 #808}.

Local U toxicity to the skin is not negligible. After application of U oxide U_3O_8 on rats for 30 days, the skin is found 50% thinner and more permeable. After 60 days, the skin remains 35% thinner and increasingly permeable compared to controls {Ubios, 1997 #768}. The mortality due to cutaneous contamination with U was also shown in rats. After U nitrate application on the skin, the diffusion of U is proportional to both the time and surface of contact. Indeed animal survival falls from 100% to 67% as rapidly as within 30 minutes of contact with 0.6 g of uranyl nitrate. After 24 hours of contact, no animal survives {Lopez, 2000 #224}.

U can also induce the secretion of inflammation proteins such as TNF alpha, and cell death, through the activation of kinase proteins such as c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) {Gazin, 2004 #809}. It can also induce apoptosis and necrosis of macrophages and CD4+ T lymphocytes. In any case, it can deregulate the immune response by modulating cytokines, even at non-cytotoxic concentrations {Wan, 2006 #810}.

For compounds enriched in U-235 isotope, radiological toxicity may occur. The radiological toxicity is predominant whatever the degree of enrichment for S compounds which are slowly transferable and express their toxicity at the site of incorporation. It is also predominant in case of chronic contaminations, except for F compounds enriched with less than 1% U-235 {Sztajnkrzyer, 2004 #1330}. This toxicity is primarily expressed in the lungs and bones, which are retention organs of such U compounds. The main radiological hazard is the occurrence of bone cancer {Wrenn, 1985 #1271}, except for poorly transferable forms that cannot reach the bones.

A recent epidemiological study related to the effect of drinking water or soil rich in U was conducted in Finland and concluded that no toxic effect could be observed in humans particularly in kidney {Kurtio, 2006 #1359}. Nevertheless, regarding the possibility of cancers emerging from U intoxication, studies conducted in France {Guseva Canu, 2011 #1358}, USA {Wagner, 2011 #1357} and Germany {Radespiel-Troger, 2013 #1356} were

much more careful on their conclusions, as data were too scarce to conclude over low toxicity of U. Nevertheless, the LD₅₀ in case of oral intake of soluble U compounds exceeds several grams of U (Table 1) {Kathren, 2008 #609}. Besides, this LD₅₀ value is probably greater for inhalation intakes of soluble U compounds. Hence, it was suggested for intakes of soluble compounds in humans that that the value of 5 g could be considered for the acute oral LD₅₀ for U and that 1 g of U for acute inhalation LD₅₀ (Table 1) {Kathren, 2008 #609}.

2.3.2. Plutonium

The toxicity of Pu is mainly due to radiation. Emission of alpha particles and neutrons (Pu-238, Pu-239, Pu-240) or beta particles (Pu-241) is the cause of a localized or global irradiation (external exposure) and/or contamination of the targeted organs after incorporation of the radionuclide into the body. Although radiation toxicity is predominant with this actinide, chemical toxicity also exists.

Regarding early and deterministic effects, the LD₅₀ were determined after injection of Pu solutions in rodents and after inhalation of Pu oxide in dogs and baboons (Table 1). Death is usually secondary to early interstitial pneumonia followed by delayed pulmonary fibrosis. The data in Table 1 show that a single inhalation of about 10 milligrams of Pu oxide is likely to cause death.

Regarding late and stochastic effects, the major observed pathologies following Pu incorporation are lung, bone and liver tumors. The incidence of pulmonary tumors was demonstrated in dog and rat after inhalation of poorly soluble compounds, such as Pu oxide. A dose-response relationship was further highlighted with a tumor onset threshold for a dose to the lungs around 1 Gy. This dose corresponds in humans to pulmonary deposition of about 200,000 Bq of Pu-239 oxide. Other non-cancerous pathologies such as pulmonary fibrosis were also observed for doses higher than 5 Gy.

However, no malignancy was observed in the lymph nodes in animals having inhaled Pu oxide. It is unlikely that Pu induces leukemia in humans. Only a single case of leukemia was observed with a dose-effect relationship in a special race of mice that received injections of Pu nitrate {Humphreys, 1987 #1332}. Experiments in dogs and rodents show that the bones

represent the main target of Pu-239 after its transfer into the blood. It may be responsible for bone tumors such as osteosarcoma with a threshold of occurrence estimated at values between 0.9 and 1.4 Gy. A recent epidemiological study shows that osteosarcoma induced by Pu preferentially localizes in the axial skeleton, in contrast to spontaneous tumors that mainly occurs at the peripheral part of the skeleton {Miller, 2003 #1316}.

Furthermore, rare liver cancers were observed in animals following inhalation or injection of oxide and various soluble forms of Pu. The induction of hepatocellular carcinoma seems to depend on the species and on the physical and chemical form of the actinide {National Council on Radiation Protection and Measurements, 2001 #796}. Finally, no hereditary disease was observed in the offsprings of contaminated animals.

3. Active chelating agents for decorporation and decontamination of actinides

In regards to external and internal human actinide contamination, it is important to provide effective chelation therapy to reduce acute radiation damage, chemical toxicity and long-term radiation effects. As proposed by different authors {Gorden, 2003 #1218; Ansoborlo, 2006 #721}, an effective actinide chelating agent should display several properties under physiological conditions such as (i) the functional groups of the chelating agent should be sufficiently deprotonated under biological medium conditions to be scavengers of the actinide of interest; (ii) site(s) of action of the chelating agent should be considered since it can affect the physicochemical properties for targeting; (iii) the agent should have high selectivity for actinides and low affinity for other biologically essential ions; (iv) low toxicity of the ligand is required; (v) the bioavailability of the chelating agent and the excretion pathways of the actinide-chelating agent complex should both be considered; (vi) the importance of the lipophilic properties of the chelating agent should be addressed; (vii) high oral efficiency is very desirable for practical application and finally, (viii) for contamination incidents, the sequestering agent should be readily available at a low cost and very quickly.

Among the different families of chelating agents, the main organic compounds and that have been synthesized and tested for their *in vivo* decorporating efficiency on actinides are given in Table 2 together with some of their important properties. The examples cited here mostly focus on U and Pu. Since the decontamination strategies have not been restricted to these two actinides, a small number of examples regarding decontamination of Am are cited in this

section. Molecules of interest comprise (i) polyaminocarboxylic acids such as diethylenetriaminepentaacetic acid (DTPA) that are effective for Pu; (ii) siderophores, such as desferrithiocin analogs, molecules using enterobactin as the ligand scaffold model, with different chelating subunits leading to numerous compounds such as catecholates (CAM), hydroxamates, hydroxypyridonates (HOPO) that are mainly effective for U and Pu; (iii) polyphosphonates with the simplest compound, 1-hydroxyethane-1,1'-diphosphonic acid (HEDP or EHBP), found to be effective for U; and (iv) macrocyclic compounds such as calixarenes displaying a high affinity for U. The chemical structure of the most important molecules are given in figure 3.

3.1. Polyaminocarboxylic acids

Polyaminocarboxylic acids (PACA) chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and DTPA, are used for the removal of some metals and radionuclides that have been incorporated into the body. Calcium salt of DTPA (Ca-DTPA) have been recognized by the scientific community as an effective tool in the treatment of internal Pu contaminations since the 1960's {Norwood, 1962 #950}. Ca-DTPA and zinc salt (Zn-DTPA) were approved by the United States Food and Drug Administration (USFDA) in 2004 for decorporation of several actinides including Pu, Am and Curium. Pharmacokinetic studies of free DTPA showed, after IV administration or inhalation, that it is not metabolized, and has a negligible tissue distribution {Stevens, 1978 #1068;Crawley, 1979 #976;Stather, 1983 #990}. In addition, it is removed relatively rapidly and mainly through the urine whatever the species (the plasma half-life is about 26 minutes in rats and increases up to 83-94 minutes in humans), and finally, retention is proportional to the body weight of the subject {Durbin, 1997 #1069}. In addition, experimental data available on the biokinetic of actinides show that the decorporating agent, to access the distribution sites of the contaminants, will have to pass the cellular and lysosomal membranes. It seems that DTPA, in its current form hardly crosses cell membranes {Taylor, 1983 #995}. This obstacle explains the fact that treatment with DTPA is really effective when administered early after the contamination when the radionuclide is still in the blood compartment or extracellular fluids.

Zn-DTPA and Ca-DTPA are used for the chelation of Pu in humans exposed to this actinide {James, 2007 #948}. Repeated daily and weekly administrations of DTPA ($30 \mu\text{mol kg}^{-1}$) for several months are recommended {Gemenetzi, 1977 #954;Guilmette, 1993 #956}. However, after such DTPA treatments the removal of 50% of the Pu is considered an exceptionally good result {Jech, 1972 #959}. Moreover, the decorporation efficacy of DTPA salts decreases after exposure since the removal of Pu deposited in the main retention sites (the liver and the bones) appears difficult due to a lack of distribution of the chelating agents in these tissues. Lung administration of DTPA as a dry powder for inhalation and delivered to humans with a Spinhaler® device has been considered {Tymen, 2000 #927}. The aerosolization performance of the micronized DTPA using a Spinhaler® is very poor with only 3% of the powder depositing in the lungs. Nevertheless, some studies in female rats have demonstrated that intrapulmonary Ca-DTPA is as effective in removing Pu that has entered the systemic circulation as intravenously administered Ca-DTPA. The IV administration of Ca-DTPA at one hour after contamination through IV injection of Pu citrate reduced the activity deposited in tissues to 43% of controls, whilst after pulmonary deposition of the same dose of Ca-DTPA, a comparable value of 40% was obtained. For soluble forms of Pu deposited in lungs the use of aerosolized Ca-DTPA can be significantly more effective than IV administration of Ca-DTPA, in reducing tissue levels of Pu {Stather, 1982 #1087}. *Ex vivo* studies in a Franz' chamber found that three successive applications for 5 minutes of a 25% solution of Ca-DTPA removes approximately 83% of the uranyl nitrate deposited on intact human skin {Tymen, 2000 #926}. However, the complexes formed between U and DTPA are unstable and the efficacy of such a treatment should be investigated carefully *in vivo* {Durbin, 2008 #711;Kullgren, 2013 #1062}.

Finally, there is widespread concern over the toxicity of DTPA. It is considered to be nephrotoxic, teratogenic, embryotoxic, and associated with suppressed hematopoiesis {Blanusa, 2005 #997}. DTPA has been shown to be associated with significant bycatch of numerous other endogenous substances (calcium, zinc, magnesium, manganese, and metalloproteinase depletion). In porcine models, U-DTPA complex caused instant cardiac arrest as a result of calcium depletion since animals provided exogenous calcium were not similarly affected by the treatment {Smith, 1966 #1049}. Inhalation of Ca-DTPA only induced

a slight histiocytosis in the lungs of treated rats. There was no significant effect of DTPA treatments on body weight, lung weight, hematology or serum chemistry values {Smith, 1980 #1075}.

A lipophilic derivative of DTPA incorporating two decane chains, called "Puchel", was synthesized to produce a chelator penetrating cells {Bulman, 1979 #1076}. Injected "Puchel" reduced liver Pu as anticipated, and when inhaled, it accelerated Pu clearance from lungs, but did not reduce the amount in the liver and was shown to be less efficient than DTPA {Stradling, 1984 #1090;Volf, 1984 #1089}. Weekly or monthly inhalations of Puchel caused lung inflammation with the finding that liver damage also occurred when it was given by injection. For this reason, its further development was abandoned {Stather, 1982 #1087}.

There are reports of DTPA having some oral efficacy {Stradling, 1993 #1066;Taylor, 2007 #1063}, but because the intestinal absorption is so low, larger quantities must be administered. Nevertheless, various partially lipophilic derivatives of DTPA having alkyl side chains of varying lengths have improved the decorporation of Pu after oral administration {Miller, 1992 #1092;Miller, 1993 #1093}. Among these compounds, docosyltriethylene tetramine pentaacetic acid (C22TT) can reduce *in vitro* the retention of Pu in the cytosol of liver cells {Bruenger, 1992 #1091}. *In vivo*, in rats, after 30 days of treatment, there were dose-related reductions in the Pu content of soft tissues and bones. All doses of C22TT resulted in substantial reductions in the Pu content of the liver, greatly reduced the incorporation of Pu into new bone and substantially reduced its content in the bone marrow {Miller, 1993 #1093}. C22TT chelators are reasonably well absorbed from the intestine and have a substantial biliary/fecal excretion pathway {Miller, 2010 #588}. Neutron-induced autoradiography studies demonstrate that the oral administration of the chelators can substantially inhibit the redistribution of Pu in skeletal tissues. In summary, C22TT chelators, having favorable bioavailability and a significant biliary excretion pathway, are efficient for Pu decorporation and are therefore good candidates for further development. More recently, a prodrug strategy using the penta-ethyl ester form of DTPA was investigated {Sueda, 2014 #1223}. Pharmacokinetic and biodistribution studies were conducted in rats after oral administration. It resulted in a sustained plasma concentration profile with higher plasma exposure and lower clearance of DTPA. In the efficacy study, rats were exposed to

aerosols of ²⁴¹Am nitrate before receiving a single oral treatment of the prodrug. The urinary excretion of ²⁴¹Am was found to be 19% higher than with the control. Consistent with prior reports related to DTPA, the prodrug was most effective when the treatment delays were minimized. Nevertheless, the most advanced results for oral delivery of DTPA concern the development of NanoDTPA[®] by the company Nanotherapeutics[®] {Reddy, 2012 #1400; Wilson, 2015 #1399}. The process consist in encapsulating within enteric-coated capsules, small particles of DTPA together with zinc acetate and optionnaly a permeation enhancer. These capsules were shown to improve significantly the oral bioavailability of DTPA in dogs {Reddy, 2012 #1400} and to display on this model similar effects as an IV administration of DTPA for the decorporation of Am-241 {Wilson, 2015 #1399}. In 2011, Nanotherapeutics[®] obtained the USFDA orphan-drug status for NanoDTPA[™] capsules to treat radiation exposure.

3.2. Siderophores

Over the years, by employing principles of biomimetic chemistry, based on the chemical structures of microbial iron transporters, called siderophores, several groups of efficient chelating agents have been identified. All are multidented ligands in which the metal binding site is at the center of the molecule. Among them, the natural product tridentate iron chelator, desferrithiocin and some analogs, enterobactin analogs, a siderophore secreted by *Escherichia coli* that comprise a catechol, and are known under the code name CAM (acronym for catechoylamide group), at last the most promising that contain hydroxypyridones (HOPO).

Due to the similarities between Fe(III) and various actinides, desferrithiocin, a potent iron chelator was used as a model for the synthesis of new chelators of actinides. A group of ten desferrithiocin analogues, consisting in tridentate ligands, were tested for their ability to reduce in contaminated rats kidney distribution of U-acetate dihydrate {Bergeron, 2009 #490}. Among these chelators, two polyether analogues increased significantly U excretion and displayed an important effect on reducing renal U content, with modest decreases in metal concentration in the bone {Bergeron, 2009 #490}. There was, however, no further clinical development of these compounds.

Within the CAM family, the most studied molecule is 3,4,3-LI-CAM (C). The suffix of CAM(C) is indicative of carboxylation of the final chelating subunit. This compound has demonstrated a promising decorporating activity for some lipophilic forms of Pu difficult to handle such as Pu complexed with tributyl phosphate (Pu-TBP) {Metivier, 1985 #1096}. This advantage is due to the very high affinity of the chelating agent for Pu (relative to that observed with DTPA) {Lloyd, 1984 #1100;Duffield, 1986 #1111}. LICAM (C) combined or not with DTPA, also has the advantage of being the only molecule capable of chelating Neptunium more effectively although the reduction is limited to 50% of the amount administered {Volf, 1986 #1118}. The significant increase in renal retention of transuranic elements induced by LICAM (C) is due to the fact that the complex actinide-LICAM is not stable at certain pH values (< 7) and dissociates in the kidneys {Durbin, 1984 #1211;Volf, 1986 #1115}. Moreover, the characteristic changes in biochemical parameters observed with LICAM (C) given alone to baboon reflected the development of a kidney disturbance and a poor general condition {Gerasimo, 1986 #1209}. These side effects have ruled out the possibility of further use of LICAM (C) in clinics. In addition, LICAM (C) was poorly effective in the decorporation of inhaled Pu {Luo, 1989 #1121;Stradling, 1989 #1117}. Other families of molecules with different chelating groups of similar structure to catecholamides, such as 5-sulfo-2,3 dihydroxybenzamide (CAM (S)) or 2,3-dihydroxyterephthalamide (TAM), were synthesized without offering significant improvement in the removal of actinides {Gorden, 2003 #1218}.

The hydroxypyridonates (HOPOs) are either tetradentate or octadentate chelators of U that have shown some promise in animal models. In this family, the most active compounds were shown to be 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) {Gorden, 2003 #1218;Ramounet-Le Gall, 2003 #1389;Durbin, 2008 #711;Abergel, 2010 #1219}. Moreover, only 3,4,3-LI(1,2-HOPO) removed potentially useful amounts of both Pu and Am from bone mineral {Guilmette, 2003 #1234}. The effectiveness of chelating U, by injection of this class of molecule, has been demonstrated *in vivo* after internal contamination of rodents {Henge-Napoli, 1995 #1212;Durbin, 2008 #711}. Indeed, IM injection of 3,4,3-LI(1,2-HOPO) immediately after contamination with uranyl nitrate reduced the retention of U in the kidneys and the femur {Henge-Napoli, 1995 #1212}. Urinary excretion of U was also increased. A 30-minutes delayed injection of 3,4,3-LI(1,2-HOPO) seemed to be less effective

against the deposition of U in bones. In addition, when the chelating treatment concentration is decreased, the ligand/U ratio becomes too low for optimal decontamination efficiency. This efficiency was also reduced if the injection was not performed immediately after contamination, even after repeated administrations {Henge-Napoli, 1995 #1212}. This efficacy was later established after IM administration to rats of 3,4,3-LI(1,2-HOPO) for reducing U, Pu and Am after IM injection of (U-Pu)O₂ particles (MOX) {Paquet, 2003 #1388}. A more recent study succeeded in removing 238-Pu citrate from mice although differences were observed between males and females {An, 2014 #1390}.

Extensive efforts have been made to develop oral delivery of 3,4,3-LI(1,2-HOPO) and the mixed ligand, 3,4,3-LI(1,2-Me-3,2-HOPO), being the first to be considered as effective agents for decorporation of Pu after oral administration {Durbin, 2003 #1228}. Subsequently, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) showed higher actinide removal efficacy after oral administration than DTPA. Toxicity studies display no negative effect on cells derived from three different human tissue sources treated *in vitro* up to ligand concentrations of 1 mM and both ligands were well tolerated in rats when orally administered daily at high doses (100 $\mu\text{mol.kg}^{-1}$ per day) over 28 days {Abergel, 2010 #1219}. No genotoxicity was demonstrated for both compounds and the maximum tolerated dose studies, performed in rats after seven consecutive days of oral administration, confirmed the safety of the efficient doses for 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) {Bunin, 2013 #1225}.

When mixed with active human liver microsomes, no loss of parent compound was observed after 60 min, indicating stability in the presence of liver microsomal cytochrome P450. At the tested concentrations, 3,4,3-LI(1,2-HOPO) did not significantly influence the activities of any of the cytochromal isoforms screened. Thus, 3,4,3-LI(1,2-HOPO) is unlikely to cause drug-drug interactions by inhibiting the metabolic clearance of co-administered drugs metabolized by these enzymes. Plasma protein-binding assays revealed that the compound is protein-bound in dogs and less extensively in rats and humans. In plasma, the compound was stable after 1 h at 37°C in mouse, rat, dog, and human plasma samples. Finally, a bidirectional permeability assay demonstrated that 3,4,3-LI(1,2-HOPO) is not permeable across the Caco-2 monolayer, highlighting the need to further evaluate the effects of various compounds with known permeability enhancement properties on the permeability of the

ligand in future studies or to design drug delivery systems to improve its oral bioavailability {Choi, 2015 #1397}.

More recently Choi et al {Choi, 2015 #1398} have evaluated the pharmacokinetics and biodistribution of the ¹⁴C-labeled 3,4,3-LI(1,2-HOPO) both in mice and rats, after administration by either IV, intraperitoneal, or oral routes. In all cases, the radiolabeled compound was rapidly distributed to various tissues and organs with differences between animal species. In mice, the 24 h fecal elimination profiles suggested that the biliary route is the predominant elimination pathway. In contrast, lower fecal excretion levels were observed in rats. The male mice eliminated a greater percentage of ¹⁴C through the renal pathway than the female mice after receiving an IV or intraperitoneal dose, while the opposite trend was seen in rats that received an IV dose. Metabolite profiling performed on selected rat samples demonstrated that a metabolite of [14C]-3,4,3-LI(1,2-HOPO) is formed, corresponding to 10% of the administered oral dose. Finally, to improve its oral bioavailability, 3,4,3-LI(1,2-HOPO) was coformulated with a proprietary permeability enhancer, leading to a notable increase in oral bioavailability of the compound. For all these reasons, in 2014, 3,4,3-LI(1,2-HOPO) received the agreement from USFDA for a phase I clinical trial.

3.3. Polyphosphonates

Organic phosphorus compounds, known for their chelation affinity with U, such as ethane 1 hydroxy 1.1. biphosphonate (EHBP), also referred to as Etidronic acid or 1-hydroxyethane 1,1-diphosphonic acid (HEDP), have also been studied for the treatment of contaminations by this radionuclide. HEDP has the advantage of already being used in clinics under the name Didronel® for the inhibition of bone resorption in the Paget's disease. It has been shown in in vivo studies in rats contaminated by with uranyl nitrate that HEDP is able, after a single injection, to avoid renal damage and to counteract the mortality due to U poisoning with a success rate of 100% {Henge-Napoli, 1999 #448}. The same molecule given orally reduced renal lesions in mice and was effective for reducing the lethal effect of U. It was at least as useful as subcutaneous administration for prompt therapy of oral U exposure, improving the

survival rate {Ubios, 1994 #1235;Martinez, 2000 #1236;Martinez, 2003 #1237;Fukuda, 2005 #1238}.

Given that bone is also a target organ for U in acute intoxication, acute exposure to U inhibits endochondral ossification, but these effects were significantly lower in animals treated with HEDP {Bozal, 2005 #1232}. Other parameters such as the oxygen-dependent erythropoietin production which is impaired by U were also counteracted by HEDP {Giglio, 1997 #1241}.

HEDP offers significant decontamination efficiency after application *ex vivo* on intact and *in vivo* on wounded skin {Tymen, 2000 #926;Houpert, 2001 #184;Houpert, 2004 #1239} contaminated with uranyl nitrate. However, if the skin is not rinsed after local application of the chelating agent, the radionuclide-HEDP complex tends to diffuse across the skin reaching deep layers and subsequently the blood {Tymen, 2000 #926}.

The most recent work regarding this family of chelators was achieved by generating a library of bisphosphonate-based ligands for U-binding {Sawicki, 2008 #693}. Twenty three dipodal and tripodal chelates bearing bisphosphonate chelating functions were found to display very high affinity for U and were selected for evaluation of their *in vivo* U removal efficacy. Among them, 11 ligands induced a huge modification of the biodistribution of U by deviating the metal from kidney and bones to liver. Among the other ligands, the most potent was a dipodal bisphosphonate which reduced the retention of uranyl and increased its excretion by around 10% of the injected metal {Sawicki, 2008 #693}. There were however no further clinical development for any of these compounds.

3.4. Calixarenes

Calixarenes have been considered as complexing agents for actinides such as U, Pu and Am for applications in the nuclear industry as they allow the selective extraction of traces of these actinides in biological matrices such as urine, or the analysis of radioactive elements in environmental samples {Bouvier-Capely, 2009 #590;Dinse, 2000 #5}.

The conformational flexibility of calix[6]arenes and the presence of functional groups facilitate the binding of metal centers {Petrella, 2004 #1242}. Among all the tested

molecules, *p*-tertbutylcalix[6]arene bearing 3 carboxylic groups arranged in C₃ symmetry with a cone conformation allows the formation of a pseudoplanar hexacoordinate complex with U. These complexes displayed a high affinity and are much more selective than calix[6]arene bearing six carboxylate groups {Araki, 1993 #1243; Dinse, 2000 #5}

Very few results have been obtained on calixarene toxicity. Some authors have claimed that the calix[6]arenes and calix[8]arenes functionalized with sulphonate groups have the same level of toxicity as glucose {Rodik, 2009 #1244}. However, a maximum of 30% of haemolysis has been observed for the sulphonated calix[8]arenes which was not the case for the calix[6]arenes {Da Silva, 2004 #1246}. The influence of the presence of carboxylic groups on the toxicity was not evaluated despite the fact that molecules such as 1,3,5-OCH₃-2,4,6-OCH₂COOH-*p*-tertbutylcalix[6]arene could be very promising for U decontamination.

4. Novel delivery systems for actinide chelating agents

4.1. Intravenous delivery systems

As U and Pu contamination concentrates in specific organs such as the liver, bones and kidneys, it was interesting to test a delivery approach consisting in liposomes. These liposomes were applied to the delivery of DTPA due to a non-relevant pharmacokinetic profile {Stevens, 1978 #1068; Crawley, 1979 #976; Stather, 1983 #990; Blank, 1984 #1214} characterized by a poor affinity for Pu major deposition sites (liver and bone) and the poor ability to cross biological membranes, resulting in a poor distribution within the body. On the contrary, lipid vesicles are able to reduce the blood clearance of the chelating agents, thus allowing a better distribution in the contaminated organs. In fact, early reports describe the use of liposomes to deliver chelating agents, particularly DTPA for the decorporation of Pu {Rahman, 1973 #1095; Rahman, 1974 #1213}. In these first reports, DTPA was encapsulated in vesicles containing egg lecithin and cholesterol. In the liposomal form, DTPA was retained longer in tissues than the free form after IV administration to mice. When given three days after Pu injection, liposomal DTPA removed a significant amount in the liver, presumably intracellular Pu that was not available to free DTPA. The urinary excretion of Pu was further increased {Rahman, 1973 #1095}. The ability of free and liposomal preparations of Ca-DTPA and Zn-DTPA to remove and reduce the body burden of actinides was confirmed by Blank *et*

al. {Blank, 1984 #1214}. In a more recent report, the encapsulation of the DTPA in multilamellar conventional liposomes composed of dioleoylphosphatidylcholine (DOPC), cholesterol (CH) and phosphatidylglycerol and in stealth® multilamellar liposomes composed of DOPC, CH and distearoyl phosphatidylethanolamine coupled to polyethylene glycol (DSPE-PEG) could modify DTPA pharmacokinetics by prolonging its circulation time and by increasing its distribution especially in the liver (conventional multilamellar liposomes) and in the bones (stealth® multilamellar liposomes). Modifications of the distribution of DTPA were well correlated with an increased decorporating effect of Pu in rats {Phan, 2004 #1215}. Their ability to remove Pu was evaluated 2 and 16 days after a single IV treatment given 2 hours after contamination with colloidal Pu (Pu-239 phytate) or with soluble Pu (Pu-238 citrate). Major increases in urine elimination together with a reduction in skeletal Pu deposition, depending of the nature of the Pu contaminant were observed {Phan, 2004 #1215}. After contamination with Pu phytate, conventional liposomes of DTPA ($6 \mu\text{mol.kg}^{-1}$) were as efficient as free DTPA ($30 \mu\text{mol.kg}^{-1}$) in maintaining the Pu content in the bones below 4.3% of the injected dose after 16 days, a 3.6-fold reduction compared with free DTPA ($4 \mu\text{mol.kg}^{-1}$) treatment or without treatment {Phan, 2004 #1215}.

Reducing the diameter of the liposomes described above to approximately 100 nm could modulate DTPA pharmacokinetic parameters by prolonging their circulation time {Phan, 2005 #1217} (Figure 4). Indeed, stealth® liposomes of around 100 nm allowed a more convenient distribution in order to improve the pharmacological effect of DTPA {Phan, 2005 #1217;Phan, 2006 #1255}. The administration of a single dose of approximately $3 \mu\text{mol.kg}^{-1}$ of DTPA encapsulated within these liposomes induced an excretion of more than 90% of the Pu after 16 days, while one dose of $30 \mu\text{mol.kg}^{-1}$ of free DTPA induced less than 50% Pu excretion under the same conditions {Phan, 2006 #1216}. Moreover, a single weekly administration of the 100 nm stealth® liposomes produced a significant reduction of Pu retention to less than 2% of the Pu injected in the liver and to less than 0.2% in the bones, only 30 days after contamination. All these improvements were attributed to the increase in the area under the curve of the DTPA pharmacokinetics and to an increased distribution of DTPA to the liver and the bones by the 100 nm stealth® liposomes. Conversely to the injection of free DTPA, in rats, 100 nm stealth® liposomes seem to be able to interact with

liver cells, then to intercept Pu and to prevent further deposition of the actinide in bones {Phan, 2006 #1216}.

4.2. Lung delivery systems

As stated above, inhalation is the main route of contamination as particles deposit throughout the airways. Whereas particles deposited in the upper airways are eliminated by the mucociliary escalator, swallowed and then excreted in the faeces, particles deposited in the alveolar region can remain for long periods of time in the lungs where they can dissolve very slowly depending on their chemical forms {International Commission on Radiological Protection, 1994 #794}. Delivering chelating agents directly to the lungs where actinides deposit after inhalation has always been considered an interesting strategy to increase local chelating agent concentrations and thus enhance decontamination efficacy. Indeed, inhalation offers the potential for needle-free, systemic delivery of small molecules {Bosquillon, 2001 #818} and would be convenient, in case of a nuclear accident, as a first pass emergency treatment instead of IV injections.

To efficiently deliver drugs in the lungs, there are basically three strategies. The first strategy consists in nebulizing a liquid solution of drug and excipients or a suspension containing the drug. The second strategy is the use of metered dose inhalers (MDIs) where the drug is adsorbed on carrier-particles such as lactose suspended in liquefied hydrofluoroalkanes. MDIs are usually used for delivering anti-asthma drugs {Colombo, 2013 #829} as they are easier to use than nebulizers. The third strategy consists in using dry powder inhalers (DPIs) where the drug is encapsulated into particles forming a powder that is delivered through a breath-activated inhaler. This last strategy proved to be attractive since it can deliver larger quantities of the drug to the lungs compared with MDIs. Powders for DPIs can be obtained by different techniques such as micronization, supercritical fluids, lyophilization or spray drying and associated techniques. The important parameter for delivering the drug to the lungs is the aerodynamic diameter of the nebulized droplets or particles exiting the nebulizer or inhaler, respectively. The aerodynamic diameter is defined as the product of the geometric diameter of the particle/droplet- its actual diameter- by the square root of the particle/droplet density {Edwards, 1997 #837;Tsapis, 2002 #922}.

Depending on their aerodynamic diameter, once inhaled particles/droplets will deposit in different regions of the lungs. The aerodynamic diameter is often given as the mass median aerodynamic diameter (MMAD). According to pharmacopoeias, one can define the fine particle fraction (FPF) which corresponds to the percentage of the administered dose of the drug actually reaching the lungs, as the dose with aerodynamic diameter below 5 μm divided by the initial dose {Cruz, 2011 #830}. Although delivering chelating agents directly in the lungs seems an obvious approach, research reports on this topic are not numerous. Two molecules have been considered so far for lung delivery: DTPA and sodium alendronate.

As detailed above, DTPA is mostly available as an injectable solution (250 mg/mL), administered by IV injection. Starting from the 1960s, research has evaluated the efficacy of directly administering DTPA as a solution in the lungs by nebulization {Tombropoulos, 1962 #918; Ducouso, 1974 #832}, showing either better or similar efficacy as the parenteral administration on pulmonary contamination, depending on the contaminant solubility. At the end of the 1990s, Japanese researchers evaluated the ability to aerosolize Ca-DTPA either as a powder or as a nebulized solution {Yamada, 1997 #938}. Ca-DTPA was aerosolized as a powder without any treatment using either a dry powder inhaler (Spinhaler®) or a dust generator (Palas model: RBG-1000). Using the Spinhaler®, the MMAD remained higher than 40 μm , indicating an unsatisfactory deposition pattern in the deep lungs. The dust generator was more efficient with MMADs around 8.5 μm . However, this value remained too high for efficient alveolar deposition. Solutions of Ca-DTPA were also nebulized (125 mg/mL or 12.5 mg/mL) leading to MMADs between 4-8 μm and 2-4 μm , respectively. These last results were very promising but no animal experiments were reported {Yamada, 1997 #938; Tymen, 2000 #927}.

Around the same time, the French army developed DTPA as a micronized dry powder for inhalation to be delivered with a Spinhaler® device {Tymen, 2000 #927}. Micronization was repeated 3 times to yield particles with a median size of 2.3 μm . The aerosolization performance of the micronized DTPA using a Spinhaler® was very poor with only 3% of the powder depositing in the alveoli. Despite its poor efficacy, this powder is available to every nuclear worker in France who could potentially be exposed to Pu.

To benefit from the improvement of inhalation technology that started in the late 19*90s {Edwards, 1997 #837;Vanbever, 1999 #930;Vanbever, 1999 #931;Cruz, 2011 #830;Ungaro, 2014 #929}, DTPA has been formulated into porous particles {Gervelas, 2007 #852}. These particles are characterized by geometric sizes larger or equal to 4-5 μm and mass densities lower than 0.4 g/cm^3 and have demonstrated high potential for drug delivery to the lungs for both local and systemic applications {Edwards, 2002 #835}. A principal advantage of porous particles relative to conventional inhaled therapeutic aerosol particles is their aerosolization efficiency {Dunbar, 1998 #834;Edwards, 2002 #836}. The delivery of large drug masses (several tens of milligrams per puff) can be obtained from a simple inhalation device {Edwards, 1997 #837}.

Particles were formulated by spray-drying DTPA (75% of final dry weight) in a mixture of ethanol-water 70/30 v/v together with 10% (final dry weight) dipalmitoylphosphatidylcholine (DPPC) added as an excipient. It is considered safe as it is the major component of lung surfactant. Leucine (15% final dry weight), an amphiphilic amino-acid that favors surface roughness and reduces particle aggregation {Vehring, 2008 #932} was also used. To favor particle porosity, ammonium bicarbonate was also added as it decomposes above 36°C into ammonia, water and carbon dioxide. Spray drying conditions were optimized to yield particles possessing a volume mean geometric diameter around $4.5 \mu\text{m}$, a crumpled-paper morphology and a powder tap density of 0.04 g/cm^3 (Figure 5). The *in vitro* aerodynamic evaluation using a multistage liquid impinger showed that about 56% of the powder deposits in the lungs (fine particle fraction) (Figure 5), with about 27% in the alveolar region (Figure 5), an improvement as compared with the marketed micronized powder available with the Spinhaler® {Tymen, 2000 #927}.

The powder was administered to rats contaminated either with Pu-nitrate or with a poorly soluble form (PuO_2). When rats were contaminated with Pu-nitrate, lung administration performed 1 hour post contamination ($22 \mu\text{mol.kg}^{-1}$ of DTPA) was 3 times more efficient than the IV administration of DTPA for reducing the Pu lung burden. In addition, Pu accumulation in the liver and bones was reduced as efficiently as with the IV treatment. These results prove that DTPA chelates Pu in the lungs and prevents Pu deposition in liver and bones. When rats were contaminated with PuO_2 and treated 1 hour post contamination,

the aerosol of DTPA was found to be as efficient as the IV administration of DTPA {Gervelas, 2007 #852; Serandour, 2007 #905; Gremy, 2012 #855}. After inhalation of Pu oxide containing various percentages of americium in rats, the epithelial lining fluid (ELF) was identified as an acellular transient pulmonary compartment, in which a fraction of actinide oxide dissolves prior to absorption and subsequent extrapulmonary deposit. Prompt pulmonary administration of DTPA as a dry powder led to a decrease in the actinide content in the ELF together with a limitation of bone and liver deposits. Because Am is more soluble than Pu, higher amounts of Am were found in the ELF, extrapulmonary tissues and urine. A higher efficacy of DTPA on americium compared to Pu in the ELF induced a preferential inhibition of extrapulmonary deposit and a greater urinary excretion of americium compared to Pu. All together, these data justify the use of an early and local DTPA treatment after inhalation of Pu oxide aerosols in which americium can be in high proportion such as in aged compounds {Gremy, 2010 #856; Gremy, 2012 #855}. This new form of inhalable DTPA therefore represents an interesting emergency treatment worth pursuing. Studies about acute and chronic toxicity of the powder should be carried out in the future.

Sodium alendronate is another interesting molecule as it can chelate U and Pu (parent radionuclides), as well as cobalt, strontium, nickel, chromium and iron (daughter radionuclides). The Mittal group has formulated alendronate into nanoparticles of about 220 nm, calling them nanosized alendronate, by means of solvent antiprecipitation {Sultana, 2014 #912}. These nanoparticles were then spray-dried to obtain a powder and mixed with lactose for delivery to the lungs. *In vitro* aerodynamic evaluation revealed that the fine particle fraction was around 58%. *In vivo*, after inhalation by healthy human volunteers about 34% of the powder was found in the lungs, with a rather homogeneous distribution as measured by scintigraphy. The pharmacokinetics of the drug after inhalation shows that the molecule crosses the pulmonary barrier, with a T_{max} at 2 hours and a $t_{1/2}$ of about 18 hours. However, this interesting formulation has not been tested for decorporation efficacy on contaminated animals. If efficient on contaminated animals, nanosized alendronate could be compared with spray-dried alendronate porous particles as proposed recently {Cruz, 2011 #830}.

There is room for major research on formulating decorporating agents for lung delivery. One immediately thinks about formulating LiHOPO into porous particles. A thorough understanding of the pharmacokinetics of decorporating agents alone and after chelation with the radionuclide would be of great interest.

4.3. Skin delivery systems

As for the other routes of exposure, contamination with U and Pu through the skin needs more attention in terms of treatment. Indeed, no specific and efficient treatment is available in case of such contamination, since only warm shower with soap and water is generally recommended {Tazart, 2013 #1260}. With the aim of limiting the transfer of U to the blood, after skin contamination, the group led by Fattal along with the French Institute for Radiological Protection and Nuclear Safety (IRSN) has developed a formulation for skin delivery incorporating the p-tertbutylcalix[6]arene bearing 3 carboxylic groups {Spagnul, 2010 #587;Spagnul, 2010 #493;Spagnul, 2010 #589;Spagnul, 2011 #661}. The formulation consists in an oil-in-water nanoemulsion where, as shown by surface tension measurements, the calixarene is positioned at the oil/water interface due to its amphiphilic character (Figure 6). The nanoemulsion prepared by the phase inversion method was obtained with 20% of liquid paraffin in which the calixarene was incorporated together with 5% of nonionic surfactants (Tween®80 and Span®80) {Spagnul, 2010 #493;Spagnul, 2010 #589} chosen for their good skin tolerance and low penetration enhancing effect. The external aqueous phase (75%) favors the elimination of this formulation by simply rinsing with water. The unloaded or the calixarene-loaded nanoemulsion were put in presence of an external aqueous phase contaminated with uranyl nitrate. The calixarene nanoemulsion allowed the extraction of most of the U compared to the unloaded nanoemulsion {Spagnul, 2010 #589}. After 5 minutes of contact, the efficacy of the calixarene nanoemulsion was optimal since 90% of the U was extracted {Spagnul, 2011 #661}. The pH of the contaminated solution remained the parameter with the greatest effect on the action of the calixarene nanoemulsion, since below a certain pH, the efficacy decreased sharply. The effect of acidification of the contaminating medium could however be minimized by the use of a nanoemulsion buffered at pH 5, a pH value that respects the skin integrity. The decontamination efficacy of the calixarene nanoemulsion was also evaluated *ex vivo* {Spagnul, 2010 #587;Spagnul, 2011

#661} using the transdermal Franz diffusion cell for 24 hours on skin from pig ear contaminated with a uranyl nitrate solution. After contamination of intact skin, although the transcutaneous diffusion of U was low due to the protective barrier formed by the *stratum corneum*, a significant decrease of 98% of the U passing through the skin was observed after immediate application of the calixarene nanoemulsion. In the case of contamination on excoriated skin, due to the absence of *stratum corneum*, the passage of U was much higher, but again a significant decrease of its diffusion was observed (about 97%) after application of the calixarene nanoemulsion {Spagnul, 2010 #587}. Comparison of the retention of U on excoriated skin treated with and without the calixarene nanoemulsion 30 minutes after contamination showed a significant decontamination efficacy of the formulation containing calixarene. 4 hours after contamination the treatment with the loaded nanoemulsion reduced by 36 times the transcutaneous passage of U compared to 4 times for the unloaded nanoemulsion. Moreover, it was interesting to note the lack of detection of calixarene in the receiver compartment after application of the formulations during 24 hours {Spagnul, 2011 #661}, indicating the potential lack of passage of the free calixarene and of the calixarene complexed with U, or indicating that the passage concerns extremely small amounts that are not detectable by the technique. Less than 0.01% of the amount of deposited calixarene may have passed through the intact and excoriated skin, this quantity being relatively negligible. To be really convincing the potential of the calixarene needs to be demonstrated *in vivo*.

Other formulations containing calixarene have also shown a decontamination effect. This is the case of a cleansing formulation consisting of a simple oil-in-water emulsion containing calixarene. The efficacy of this emulsion was compared with two other reference treatments consisting of DTPA and HEDP solutions. Application of the calixarene emulsion induced the highest decontamination effect with an 87% decrease in U diffusion flux. By contrast, the HEDP and DTPA solutions only allowed a 50% and 55% reduction of U permeation, respectively, and had the same effect as a simple dilution of the contamination with pure water {Phan, 2013 #738}. Nanoemulsions, however, might not be the most suitable formulation for topical delivery due to their liquid behavior. In order to modulate their viscosity, a hybrid formulation composed of the nanoemulsion described above and a hydrogel was formulated. It was shown to be as efficient as the plain nanoemulsion to treat

U skin contamination, while application on skin was made easier {Belhomme-Henry, 2014 #746}.

Very recently, a French company named Medesis® produced a water-in-oil emulsion comprising a complex nanometric self-structured micellar system called Aonys® {Mouri, 2014 #1402;Mouri, 2014 #1403}. Medesis® claims Aonys® can be applied for the delivery of metal chelating molecules despite they did not publish any paper on this particular application. Whether, this formulation could be used for skin delivery of chelating agents remains to be studied.

5. Conclusion

Contamination with actinides such as U and Pu can lead to severe damages for exposed workers or civilians whether they are contaminated through the skin, lungs or systemically. Only a small number of drugs have been clinically developed for treating such contaminations. Among these drugs, very clearly DTPA and HOPOs have been the best candidates for clinical application, the first being used for quite a long time and the second being, with 3,4,3-LI(1,2-HOPO), quite close to clinical testing. Nevertheless, it is important to keep in mind that when dealing with local exposure such as skin and lungs, the principles should be to design a delivery system that prevent further diffusion of the actinide in blood. When dealing with systemic contamination, one of the strategy is to administer orally the chelating agent. In this case, it is very important to imagine possibilities of absorption enhancers, either molecular or colloidal, to increase the bioavailability of the chelating molecules. Despite the great advantage of oral formulation, it should be of high importance to imagine drug targeting strategy where the active drug pharmacokinetics and tissue distribution would follow the actinide biokinetics. This should be the best strategy to reduce as much as possible the actinide tissular and cellular content. As shown in this review, “smart” delivery systems demonstrate in all cases a real advantage compared to conventional therapy independently of the route of administration and the number of delivery systems utilized for such purpose should increase in the future.

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Table 1: Lethal doses causing the death of 50% of individuals (LD₅₀) in animal or humans contaminated by different physicochemical forms of U, Pu-238 or Pu-239.

Isotope	Species	Mode of contamination	LD ₅₀	Time interval*	References
U	Human	Oral	5 g	-	{Kathren, 2008 #609}
		Soluble compounds inhalation	1 g	-	{Kathren, 2008 #609}
Pu-238	Rat	Citrate injection	6.0 MBq.kg-1 (9.5 µg.kg-1)	30 days	{Mahlum, 1969 #1318}
		Nitrate injection	3.6 MBq.kg-1 (5.7 µg.kg-1)	30 days	{Mahlum, 1969 #1318}
Pu-239	Rat	Citrate injection	3.6 MBq.kg-1 (1.6 mg.kg-1)	30 days	{Mahlum, 1974 #1321}
		Nitrate injection	1.7 MBq.kg-1 (0.74 mg.kg-1)	30 days	{Mahlum, 1974 #1321}
	Human**	Oxide inhalation	21 MBq (9.0 mg)	30 days	{Beir IV, 1988 #1331}
			2.1 MBq (0.9 mg)	365 days	{Beir IV, 1988 #1331}
			0.85 MBq (0.4 mg)	1000 days	{Beir IV, 1988 #1331}

* This time interval corresponds to the time at which the count of dead animals was made

** Estimation from animal data (dog, baboon)

Table 2: Main families of ligands tested for decontamination/decorporation of Pu and U

Ligand family	Chelating agents	Elements	Dose given	Toxicity or side effects	State of clinical development
Polyaminocarboxylic acids	DTPA Ca, Zn, Na	Pu(IV)	1g by iv or inhalation for 6 days in humans 30 $\mu\text{mol.kg}^{-1}$ and 3,2 $\mu\text{mol.kg}^{-1}$ in liposomal form in rats	Nephrotoxicity, teratogenicity, embryotoxicity, suppressed hematopoiesis	In clinics by parenteral and lung administration
Siderophores	3,4, 3-LI-CAM(S) or 3,4,3-LI-CAM(C).	Pu(IV),	30 $\mu\text{mol.kg}^{-1}$ in dogs,	Nephrotoxicity	Preclinical
	3,4,3-LI(1,2-HOPO)	Pu(IV), U(VI), can be applied to other actinides	30 $\mu\text{mol.kg}^{-1}$ oral or iv in mice	Low acute toxicity in mice and well tolerated at high doses in rats (100 to 150 $\mu\text{mol.kg}^{-1}$ per day over 28 days)	Phase I clinical trial
	5-LIO(Me-3,2-HOPO)	Pu(IV), U(VI), can be applied to other actinides	30 $\mu\text{mol.kg}^{-1}$ iv or oral in mice	Low acute toxicity in mice and well tolerated at high doses in Rats (100 to 150 $\mu\text{mol.kg}^{-1}$ per day over 28 days)	Preclinical
Polyphosphonates	HEDP or EHBP	U(VI)	50-100 $\mu\text{mol.kg}^{-1}$	Nephrotoxicity, Serum calcium drop	In clinics (DidroneI®) used to strengthen bone, treat osteoporosis, and treat Paget's disease
Macrocycles	para-tertio-butyl-calix[6]arene	U(VI)	Not tested in vivo	Not tested	Preclinical

Captions to figures

Figure 1: Frequencies of different modes of contamination by actinides that occurred in French CEA-AREVA centers between 1970 and 2003. Adapted from {Grappin, 2007 #1267}

Figure 2: Biokinetic model of radionuclides contaminations

Figure 3: Chemical structure of major chelating agents

Figure 4: Pharmacokinetics in mice of DTPA administered intravenously in solution or entrapped in long circulating properties. Adapted from {Phan, 2004 #1215}.

Figure 5: Characteristics of the optimized powder of DTPA. On the left, aerodynamic properties of DTPA powder. On the right, image of one DTPA particle by scanning electron microscopy. Adapted from {Gervelas, 2007 #852}.

Figure 6: Insertion of 1,3,5-OCH₃-2,4,6-OCH₂COOH-*p*-*tert*butylcalix[6]arene into the nanoemulsion shell.

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