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Compared *in vivo* efficiency of nanoemulsions unloaded and loaded with calixarene and soapy water in the treatment of superficial wounds contaminated by uranium

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ABSTRACT

No emergency decontamination treatment is currently available in the case of radiological skin contamination by uranium compounds. First responders in the workplace or during an industrial nuclear accident must be able to treat internal contamination through skin. For this purpose, a calixarene nanoemulsion was developed for the treatment of intact skin or superficial wounds contaminated by uranium, and the decontamination efficiency of this nanoemulsion was investigated *in vitro* and *ex vivo*. The present work addresses the *in vivo* decontamination efficiency of this nanoemulsion, using a rat model. This efficiency is compared to the radio-decontaminant soapy water currently used in France (Trait rouge®) in the workplace. The results showed that both calixarene-loaded nanoemulsion and non-loaded nanoemulsion allowed a significant decontamination efficiency compared to the treatment with soapy water. Early application of the nanoemulsions on contaminated excoriated rat skin allowed decreasing the uranium content by around 85% in femurs, 95% in kidneys and 93% in urines. For skin wounded by microneedles, mimicking wounds by microstings, nanoemulsions allowed approximately a 94% decrease in the uranium retention in kidneys. However, specific chelation of uranium by calixarene molecules within the nanoemulsion was not statistically significant, probably because of the limited calixarene-to-uranium molar ratio in these experiment conditions. Moreover, these studies showed that the soapy water treatment potentiates the transcutaneous passage of uranium, thus making it bioavailable, in particular when the skin is superficially wounded.

1. Introduction

Skin contamination and inhalation are the most frequent contamination routes in the nuclear industry. Cutaneous contamination by radionuclides, such as uranium compounds on intact or wounded skin, is a significant source of exposure [1], and may lead to incorporation of actinides into the body through the bloodstream. Currently, there is no treatment for the prevention of percutaneous passage of diffusible uranium, such as uranyl ions [2]. Such radionuclides can pass through intact skin in less than fifteen minutes and diffuse to the bloodstream within thirty minutes of the contamination event [3]. Numerous studies have shown that the transcutaneous diffusion of uranium is conditioned by the skin integrity, since lesions of the *stratum corneum* induce an increased bioavailability of the uranium [3–5]. Thus, an initial external contamination will induce internal exposure of the victim to uranium, some of which will be directly eliminated by urinary excretion, whereas the remainder will be stored in retention organs, i.e. kidneys and bones [6–9]. This uranium retention in the target organs can cause both chemical and radiological toxicities [10–14].

In practice, in case of cutaneous contamination, the only treatment currently available in the French nuclear industry consists in rinsing the contaminated area with tepid water and soap [15,16]. However, this procedure is not specific for trapping radionuclides.

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Indeed, there is no efficient chemical or anatomical treatment against cutaneous uranium contamination, despite the fact that contamination can occur through wounds that are difficult to decontaminate. If there is suspicion of internal contamination after cutaneous contamination, one or several intravenous injections of a Ca-DTPA solution can be performed after transfer of the victim to a medical department, in order to promote the urinary excretion of radionuclides such as transuranic actinides [17]. However, this decorporating agent is not efficient for specific chelation of uranium in the body, and may adversely potentiate its nephrotoxic effects [17,18].

A local treatment strategy, directly applied on the contaminated area, seems to be the most effective way to reduce the absorption of radionuclides after cutaneous contamination [19]. Several previous studies have been conducted to this end, especially leading to the development of hydrogel treatments. The authors demonstrated a significant decrease of renal uranium uptake thanks to the retention of the radionuclide within the hydrogel at the contaminated sites [20,21]. However, addition of specific chelating agents was not found to improve the gel's decontamination efficiency [20,21], or could even reduce the gel's decontaminating effect [22]. Indeed, the observed efficiency resulted in a non-specific physical absorption of the contaminated solution in the formulations [22], which may induce release of uranium from the gels at any time. Specific chelating agents, particularly phosphorus organic compounds, such as ethane-1-hydroxy-1,1-bisphoshonate (EHBP), have been investigated for the treatment of uranium cutaneous contamination [23–25]. Ex *vivo* and *in vivo* studies found that EHBP is a more efficient decontaminating agent than DTPA after a local treatment following a contamination accident with uranyl nitrate solution. However, it appeared that if the skin was not rinsed after the local application of the chelating agent, the radionuclide-EHBP complexes diffuse towards the lowest skin layers in larger proportions than the DTPA-radionuclide complexes. This diffusion phenomenon could lead to a distribution of the complex in the bloodstream [26]. In other studies, other bisphosphonate molecules, such as pamidronate, were used as uranyl ion ligands and were incorporated in hydrogels [21]. All formulations showed decontamination efficiency but no clear effect of the chelating agents was demonstrated as no hydrogels without chelating agents were tested as control treatments. This efficiency could be due to the absorbing capacity of the hydrogel. Consequently, there is no actual and efficient treatment for uranium cutaneous contamination.

In this context, we developed a new emergency treatment to be used in case of uranium cutaneous contamination, consisting of an oil-in-water (O/W) nanoemulsion comprising calixarene molecules [27], a specific chelating agent of uranium [28–30]. Previous studies on *ex vivo* models, using intact and superficially wounded and contaminated skin explants, demonstrated the efficiency of this formulation [31,32]. The cutaneous safety of this calixarene nanoemulsion was also demonstrated by *in vitro* studies on reconstructed human epidermis [32].

The aim of the present study was to evaluate the *in vivo* decontamination efficiency of this calixarene nanoemulsion. For this purpose, rat models were used and contaminated on their back skin after being superficially wounded by excoriation or stings. Indeed, internal contamination by actinides following wounding may occur in nuclear industry workers or subsequent to terrorist activities [33]. On this animal model, the decontamination efficiency of our calixarene nanoemulsion was compared to the reference radio-decontaminant treatment used in the French nuclear industry, i.e. Trait rouge®, hereafter referred to as soapy water.

2. Materials and methods

2.1. Chemicals

1,3,5-OCH_3-2,4,6-OCH_2COOH-*p*-*tert*butylcalix [6]arene, hereafter referred to as calixarene (Fig. 1) was synthesized as described in the patent [34]. Other compounds used for preparing the calixarene nanoemulsions were paraffin oil (d = 0.86, VWR, Fontenay-sous-Bois, France), non-ionic surfactants sorbitan monooleate (Span® 80) and polyoxyethylene glycol sorbitan monooleate (Tween® 80), both purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Demineralized water was obtained from a Milli-Q® Synergy 185 water purification system (Millipore, Saint-Quentin-en-Yvelines, France).

Uranium-contaminated solution was prepared from depleted uranium (1000 mg L^{-1} in 2% HNO_3) purchased from SPEX CertiPrep, HORIBA Jobin Yvon (Longjumeau, France), to a final concentration of uranium at 0.04 g L^{-1} pH 5. The isotopic composition was 0.0013% of ^{234}U, 0.3% of ^{235}U and 99.6987% of ^{238}U. The digestion of organs was carried out with 67% HNO_3 stock solution and 30% H_2O_2 (VWR, Fontenay-sous-Bois, France), using the ETHOS One® microwave oven (Milestone Srl, Sorisole, Italy). The following reagents and standards were used for ICP-MS measurements: 67% HNO_3 stock solution (Normatom®, VWR, Fontenay-sous-Bois, France) and a spike isotopic reference material containing ^{233}U and ^{236}U with a certified amount ratio *n* (^{233}U)/*n* (^{236}U) of 1.01906 (16) was purchased from Institute for Reference Materials and Measurements (IRMM) (Geel, Belgium).

2.2. Preparation and characterization of calixarene-loaded nanoemulsion

The emulsion inversion point method was used to prepare the O/W calixarene nanoemulsion as previously described [27]. Briefly, water was added dropwise into a mixture of paraffin oil (20%) and surfactants (5%) under stirring at 50 °C. Calixarene was added to the oily phase at a concentration of 4 mg g^{-1}. The dispersed oil droplet size and zeta potential were both measured with a Nanosizer/Zetasizer® ZS apparatus (Malvern Instruments, Worcestershire, UK), and pH measurements were carried out with a MeterLab/pH M240 pH-meter (Radiometer, Copenhagen, Denmark).

2.3. In vivo studies

2.3.1. Animals

Three-month-old male Sprague Dawley rats purchased from Charles River Laboratories (Saint Germain sur l'Arbresle, France) were handled according to the French Legislation (articles R214-87 to R214-137 of Rural Code) and the European Directives (2010/63/EU) regarding the care and use of laboratory animals. Experimental protocols were validated by the IRSN Ethics Committee. At least six

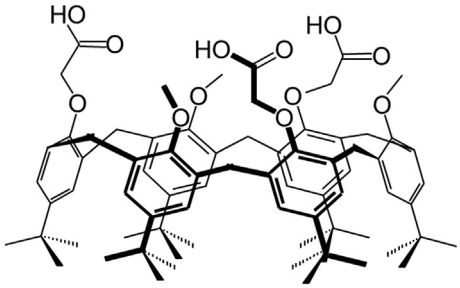

Fig. 1. Structure of 1,3,5-OCH3-2,4,6-OCH2COOH-*p*-tertbutylcalix [6]arene.