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Research Paper

X-ray microfluorescence for biodistribution studies of nanomedicines

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ABSTRACT

Currently, the *in vivo* distribution of drugs is investigated by non-spatial quantitative techniques. With the emergence of personal therapies using nanomedicines, deeper investigations are required to precisely know the *in vivo* fate of entrapped drugs, especially to predict possible toxicity. Here, we assess the capabilities of SR-μXRF for *i*) detecting drugs into nanomedicines without adding any marker, *ii*) mapping their distribution into tissues and *iii*) locally quantifying the drugs loaded into nanomedicines. To prepare the nanomedicine model, we used the bioconjugate diamine(dichloro)platinum (SQ-CDD) developed in the TERNANOMED Grant Project. Nanomedicines were *intravenously* injected into a nude mice model bearing a pancreatic tumour (PANC-1). The X-ray microfluorescence experiments were performed on embeds tissue sections of kidney and tumor at 2 h and 24 h after nanoparticles injection. Data collection was performed on the micro-imaging beamline ID13 of the European Synchrotron Radiation Facility (ESRF). A quantitative study was performed by atomic absorption spectroscopy (AAS), allowing to compare the platinum concentrations with those measured by X-ray. This study shows that the synchrotron radiation-based μXRF analysis is sensitive enough to detect and map the distribution of a drug entrapped into nanomedicine. A quantitative local analysis is possible with a tissue element as reference, or semi-quantitatively if the tissue reference is not homogenous.

1. Introduction

The efficacy of conventional drugs is limited by non-specific biodistribution, causing significant side effects, especially in the treatment of cancers, as it is the case with diammine(dichloro)platinum (CDDP), a major anticancer compound (Broomhead et al., 1980). The introduction of nanotechnology into pharmacology through the use of nanomedicines represents a promising opportunity to overcome the toxicity of anti-cancer drugs (Kreuter, 1994). These nanocarriers can be solid lipid nanoparticles (NPs), liposomes or biodegradable and biocompatible polymeric NPs (Faraji and Wipf, 2009). The effectiveness of the encapsulated drug may be improved, due to a better targeting of cancer cells and tissues, also avoiding tumor escape (Arias et al., 2011; Brigger et al., 2002). Because of their poorly predictable nature, any novel nanomedicine requires full physicochemical and biological characterizations. However, entrapping a drug into a nanoformulation may also lead to new types of toxicity during pre-clinical or clinical developments, different from those observed in classical galenic formulations. Thus, biodistribution studies are of importance also to guide toxicological assays.

Currently, the biodistribution of drugs is investigated by global analytical techniques such as high performance liquid chromatography (HPLC), inductively coupled plasma mass spectrometry (ICP-MS) (El-Khateeb et al., 1999), Raman spectroscopy (Stiebing et al., 2014) and atomic absorption spectroscopy (AAS) (Simpson et al., 2013). These analytical methods present the major inconvenience to only provide global quantification. When dealing with nanomedicines, it is necessary to obtain a more precise information about the distribution inside a given organ, which therefore requires spatial resolution and high sensitivity. More details concerning the NPs biodistribution study is particularly needed in the present pharmaceutical context where regulation is hardening market access (Afssaps, 2011). Many imaging techniques, grouped under the term of preclinical imaging can address this problem, either *ex vivo* (tissue or cell imaging) or *in vivo*. The location of the therapeutic molecule delivered by the nanomedicines is, indeed, very important for a better understanding of their

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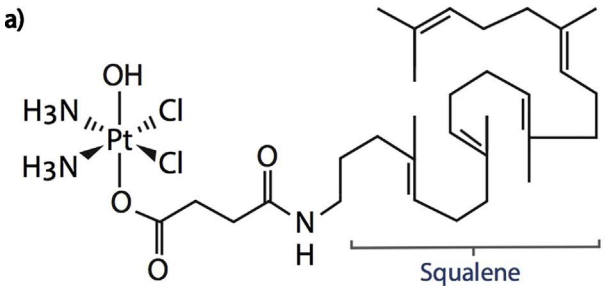

Fig. 1. (a) Structure of the squalene diammine(dichloro)platinum bioconjugate (SQ-CDDP) (b) image of SQ-CDDP NPs observed by cryotransmission electron microscopy (cryoTEM).

pharmacological activity and delineates the distribution differences with the more conventional delivery modes.

The techniques for imaging the biodistribution of drugs can be divided into two main groups. The first comprises several non-invasive *in vivo* analyses but are characterized by a rather poor spatial resolution (mm range) and by the requirement for an exogenous marker. These techniques are very efficient for medical diagnosis at the organ level, to detect and localize drugs in pathogen areas. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) necessitate a radionucleotide marker (Buck et al., 2009; Chan et al., 2002; Schottelius and Wester, 2009) and optical fluorescence imaging a fluorophore (Rao et al., 2007). Echography can be carried out using microbubble as marker to follow the drug biodistribution (Liu et al., 2006) and MRI a magnetic contrast agent such as iron oxide particles or Gadolinium chelates (Cole et al., 2011; Park et al., 2008). The second group consists of several high-resolution (≤ µm range) techniques, but is only practicable on *ex vivo* dissected tissues. No marker is required, representing the main benefit of these 2D techniques. FT-IR (Miller and Dumas, 2006; Miller et al., 2006) and UV microscopies (Petit et al., 2010) are sensitive to the nature of the chemical bonds, whereas nanoscale secondary ion mass spectrometry (Nano-SIMS) (Grovenor et al., 2006) and synchrotron radiation-induced micro X-ray fluorescence spectrometry (SR-µXRF) are sensitive to the chemical elements (Bussy et al., 2008). The spatial resolution of Nano-SIMS (50 nm) is better than that of SR-µXRF (500 nm), however its sensitivity is lower and all elements are not detectable, unlike for SR-µXRF. In view of its high sensitivity to detect low-concentrated elements, SR-µXRF represents a promising technique to detect and analyse drugs in tissues and to investigates their biodistribution, without any prior marking labelling. SR-µXRF has been successfully used for biomedical driven studies dealing with tissues like hair (Mérigoux et al., 2003), brain (Davies et al., 2015; Miller et al., 2006), bone (Ballarre et al., 2013; Brock et al., 2013) and for the detection of contaminants in vegetal samples (Larue et al., 2012), ecology (Feng et al., 2015). It has also been used to map the element distribution in cell culture (Kosior et al., 2012; Ortega et al., 2007).

Several studies using imaging methods that require a marker have been conducted to study the biodistribution of drugs encapsulated into nanoparticulate formulations, like a fluorescent (rhodamine) (Semete et al., 2010) or a magnetic marker (Arias et al., 2011). Our aim was therefore to assess the capabilities of SR-µXRF for detecting nanomedicines in *ex vivo* tissues without the use of any marker. This approach represents an interesting alternative to the so-called theranostic approach.

The questions addressed in the present study were as follows:

i Is the sensitivity of the technique high enough in view of the low amount of the injected drug (ppm range), in particular in the targeted tumors?
ii Is it possible to map the distribution of nanomedicines in tissues?
iii Is a semi-quantitative or quantitative analysis of the local concentration of nanomedicines possible?

The choice of the model nanomedicine to test the ability of SR-µXRF was guided by two criteria: the presence of an atom giving a well identified fluorescence signal and an innovative biodegradable nanocarrier with an interesting pharmacological activity. The selected drug was the diammine(dichloro)platinum (CDDP), a well-known anticancer drug used in many cancer treatments (Kelland, 2007) but leading to pronounced resistance and side-effects (Siddik, 2003). CDDP is a good candidate for X-ray fluorescence analysis because platinum (Pt) is absent from living tissues and is expected to give at least one signal of fluorescence, well separated from those of the natural atoms present in the biological tissues. Aiming at reducing the resistance and side effects, CDDP was covalently linked to a squalene derivative through a succinate spacer, forming a bioconjugate (SQ-CDDP) which spontaneously self-assembled into nanoparticles of 100–200 nm displaying an impressive anticancer activity in various colo-rectal cancers (Kotelevets et al., 2017). Of note, the so-called “squalenoylation” discovered ten years ago (Couvreur et al., 2006), exhibits a high potential in drug delivery since many drug molecules can be incorporated after chemical grafting to the squalene derivative, like anticancer, antibiotic, anti-ischemic or anticoagulant drugs (Abed et al., 2015; Arias et al., 2011; Gaudin et al., 2014; Maksimenko et al., 2014; Ralay-Ranaivo et al., 2014). It is now clearly identified that the squalene moiety of those bioconjugates is using the natural cholesterol rich lipoproteins as indirect carriers to target diseased tissues and cells (Sobot et al., 2017a,b) In the present study, we show that SR-µXRF may represent a new and useful approach to investigate the biodistribution of cisplatin containing nanoparticles when injected intravenously into mice bearing a PANC-1 tumor.

2. Materials and methods

2.1. Nanomedicine preparation

The synthesis of SQ-CDDP bioconjugate depicted in Fig. 1 was achieved from hydroxo succinate *cis*-diammine(dichloro)platinum (Dhar et al., 2009) by coupling with trisnor-aminosqualene in 25% yield after chromatographic purification. The SQ-CDDP NPs were prepared following the nanoprecipitation-evaporation method. Briefly, the SQ-CDDP bioconjugate was dissolved in an ethanol solution at 6.5 mg/mL concentration. This organic phase was then added dropwise under mechanical steering at room temperature (25 °C) in an aqueous phase containing 5% dextrose (w/v), leading to the spontaneous self-assembly process into nanoparticles. The organic solvent was afterwards evaporated under reduced pressure using a Rotavapor® at 37 °C. The average diameter of the SQ-CDDP NPs was 128 nm, with 0.1 of polydispersity index and a Zeta potential of −49 mV.

2.2. Preparation of ex vivo samples

Drugs were injected intravenously in nude mice bearing subcutaneously grafted human PANC-1 tumor. The intravenously injected doses were 2 mg/kg eq. Pt for SQ-CDDP NPs and 3 mg/kg eq. Pt for Cisplatin drug, corresponding to the maximum tolerated dose (MTD) for each treatment. Two sets of mice were prepared, corresponding to two time intervals between injection and sacrifice (2 and 24 h). Animals