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Albumin-bound nanoparticles of practically water-insoluble antimalarial lead greatly enhance its efficacy

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ABSTRACT

We recently showed that the indolone-*N*-oxides can be promising candidates for the treatment of chloroquine-resistant malaria. However, the *in vivo* assays have been hampered by the very poor aqueous solubility of these compounds resulting in poor and variable activity. Here, we describe the preparation, characterization and *in vivo* evaluation of biodegradable albumin-bound indolone-*N*-oxide nanoparticles. Nanoparticles were prepared by precipitation followed by high-pressure homogenization and characterized by photon correlation spectroscopy, transmission electron microscopy, differential scanning calorimetry and X-ray powder diffraction. The process was optimized to yield nanoparticles of controllable diameter with narrow size distribution suitable for intravenous administration, which guarantees direct drug contact with parasitized erythrocytes. Stable nanoparticles showed greatly enhanced dissolution rate (complete drug release within 30 min compared to 1.5% of pure drug) preserving the rapid antimalarial activity. The formulation achieved complete cure of *Plasmodium berghei*-infected mice at 25 mg/kg with parasitemia inhibition (99.1%) comparable to that of artesunate and chloroquine and was remarkably more effective in prolonging survival time and inhibiting recrudescence. In 'humanized' mice infected with *Plasmodium falciparum*, the same dose proved to be highly effective: with parasitemia reduced by 97.5% and the mean survival time prolonged. This formulation can help advance the preclinical trials of indolone-*N*-oxides. Albumin-bound nanoparticles represent a new strategic approach to use this most abundant plasma protein to target malaria-infected erythrocytes.

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1. Introduction

The constant rise of malaria parasite resistance to currently established antimalarials is a challenging hurdle. Resistance to artemisinins (the first line therapy of uncomplicated malaria) has spread to new locations in Asia, out of the Greater Mekong subregion (Cui et al., 2012). This alarming situation implies the development of new antimalarial structural archetypes (Murray et al., 2012; White, 2010). Paradoxically, no new antimalarial molecular scaffolds have been introduced into clinical practice since 1996 (Gamo et al., 2010). Recently, we have developed a series of indolone-*N*-oxides (INODs) with potent and rapid antimalarial activity (Nepveu et al., 2010). This series afforded a new antimalarial molecular scaffold and represented promising drug candidates for areas where chloroquine-resistant *Plasmodium falciparum* strains prevail (Nepveu et al., 2010; Tahar et al., 2011). We demonstrated the rapid uptake and biotransformation of INODs into an active metabolite in human erythrocytes (Ibrahim et al., 2011). We also showed that INODs, through a mechanism triggered by the activation of a redox signalling pathway, cause a membrane

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destabilization and vesiculation of the *P. falciparum*-infected erythrocytes which in turn become not only less hospitable for parasite maturation, but also less mechanically stable and capable of harbouring the developing parasite (Pantaleo et al., 2012). When we moved forward in the drug discovery process, the *in vivo* assays of these compounds (especially the toxicity studies where high concentrations of the substance are used) have been hampered by their very poor aqueous solubility (<10 μg/mL). It is to be noted that in spite of the diverse biological properties of INODs, including anti-infectious (Hooper et al., 1965; Sahasrabudhe et al., 1980; Ibrahim et al., 2012), neuroprotective (Menton et al., 2002), inhibitors of mitochondrial ATP synthesis (Foster et al., 1983), and reactive oxygen species scavengers (Ramana et al., 2010; Boyer et al., 2004), limited data are available on their *in vivo* activities (Menton et al., 2002). Poor solubility is a major challenge in drug discovery and is often cited as a main reason for the discontinuation of the development of many new chemical entities since 60% of drugs coming directly from synthesis are facing solubility problems (Muller and Junghanns, 2006; Kumar and Burgess, 2012). It was clear to us that for the *in vivo* evaluation of these compounds, suitable formulations were required to enable the intravenous route of administration to be used. This guarantees direct delivery of antimalarial drug to the site of infection (blood).

Among the different formulation strategies used to deliver poorly soluble drugs, micronization is a classic approach that enhances the dissolution rate by a significant increase in the surface-to-volume ratio. Nevertheless, if the compound has a very poor aqueous solubility, *e.g.*, below the mg/mL level, size reduction by micronization is not sufficient (Muller and Keck, 2004). Furthermore, microparticles of diameter greater than 5 μm are not suitable for intravenous administration since they can lead to capillary blockage and emboli when they exceed a critical level in the administered dose (Schroeder et al., 1978). Recently, nanonization has been introduced to achieve particle sizes between 10 nm and 1000 nm (Muller and Keck, 2004). Nanonization not only increases the dissolution rate, but also simultaneously the saturation solubility (Muller and Keck, 2004; Gao et al., 2008). Nanosuspensions can be injected parenterally, especially intravenously, which, by definition, leads to 100% bioavailability. Various macromolecular substances have been used for the preparation of biodegradable nanoparticles. Among these, human serum albumin (HSA) has proved to be a promising drug carrier due to its endogenous, non-toxic and non-immunogenic properties (Desai et al., 1999). Albumin is known as a natural carrier of hydrophobic molecules that allows the transport of these molecules around the body and their release at the cell surface (Elzoghby et al., 2012). We have recently reported the encouraging binding affinities of INODs towards HSA (Ibrahim et al., 2010), which may provide a basis for their rational use in clinical practice. The use of HSA-bound nanoparticles of the antimalarial INODs is an intelligent strategic approach since HSA is taken up and degraded selectively by *P. falciparum*-infected erythrocytes where it may serve as an additional source of amino acids, along with haemoglobin (El Tahir et al., 2003; Duranton et al., 2008). Furthermore, albumin is the only adjunctive therapy that decreases mortality in severe and cerebral malaria cases (John et al., 2010). Additionally, biodegradable nanoparticles seem to be promising carriers to increase drug circulation time along with the degradation of the polymer (Ishihara et al., 2008). As no other surfactants or polymeric materials are used, albumin-bound nanoparticles are well-tolerated. Despite being an ingenious concept, few nanoparticle formulations have reached the pharmaceutical market, though albumin-bound nanoparticles of the anticancer drug paclitaxel (Abraxane®) were approved by the United States Food and Drug Administration (FDA) in 2005 for the treatment of metastatic breast cancer (Ibrahim et al., 2002).

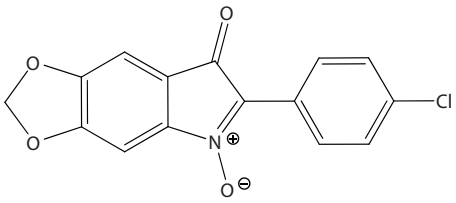

Fig. 1. Chemical structure of the drug tested, 6-(4-chlorophenyl)-7*H*-[1,3]dioxolo[4,5-f]indol-7-one-5-oxide.

The objective of the present study was to develop an albumin-bound nanoparticle formulation of INODs, suitable for intravenous administration and preserving the rapid action of the drug, based on a combination of a bottom-up process (typically precipitation) followed by a top-down technique (high pressure homogenization, HPH). From the series of INODs, we tested 6-(4-chlorophenyl)-7*H*-[1,3]dioxolo[4,5-f]indol-7-one-5-oxide (Fig. 1). This compound (referred to as "drug" in the present text) was selected as it had the best profile for *in vivo* antimalarial assays (Nepveu et al., 2010). A systematic investigation of parameters influencing the effectiveness of size reduction is also presented. The nanoparticles (NPs) were characterized in terms of their diameter and polydispersity index (PI) by photon correlation spectroscopy (PCS) and zeta potential by electrophoretic light scattering (ELS). The nanoparticle morphology was examined by transmission electron microscopy (TEM). The existing form of the drug in the nanoparticles was inspected by differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). *In vivo* antimalarial activity was evaluated using a murine model infected with *Plasmodium berghei* and with 'humanized' mice infected with *P. falciparum*. Our aim was to develop an albumin-bound drug nanoparticle formulation compatible with the intravenous route and preferentially with the minimum achievable particle size to provide nanoparticles targeting red blood cells and to improve drug dissolution, along with the maximum homogeneity (polydispersity index < 0.25), which favours good physical stability and safety.

2. Materials and methods

2.1. Materials

The drug: 6-(4-chlorophenyl)-7*H*-[1,3]dioxolo [4,5-f]indol-7-one-5-oxide (MW = 301.7 g mol^{-1}; log *P* calculated with VCCLAB[1] = 2.09) was synthesized in our laboratory as previously reported (Nepveu et al., 2010). Acetone (HPLC grade), phosphate buffered saline (PBS) tablets and HSA (essentially fatty acid free; MW = 66 478 g mol^{-1}) were purchased from Sigma–Aldrich (St. Quentin, France). All aqueous solutions were prepared using high-purity distilled water obtained from a Milli Q® water purification system (Millipore, St. Quentin, France). Dissolution of compounds was enhanced by sonication in an ultrasonic bath (Elma®, Germany).

2.2. Preparation of physical mixture and drug/HSA nanoparticles

A physical mixture of the drug and HSA was prepared in a ratio of 1:5 (w/w) by geometric dilution. 10 mg of the drug was thoroughly mixed firstly with 10 mg HSA, then 20 mg HSA was added and mixed and the remaining 20 mg HSA was finally added.

Drug/HSA nanosuspensions containing a drug concentration of 0.5, 1, 2.5 and 10 mg/mL were prepared by precipitation followed by

[1] Log *P* calculated with VCCLAB (http://www.virtuallaboratory.org/lab/alogps/start.html).

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