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Artemisinin nanoformulation suitable for intravenous injection: Preparation, characterization and antimalarial activities

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ABSTRACT

More than 40 years after its discovery, artemisinin has become the most promising antimalarial agent. However, no intravenous formulation is available due to its poor aqueous solubility. Here, we report the preparation, characterization, and *in vitro* and *in vivo* biological evaluation of biodegradable albumin-bound artemisinin nanoparticles. The nanoparticles were prepared by a combination of a bottom-up and a top-down processes and characterized by different spectroscopic techniques. The preparation process was optimized to develop a nanoformulation with the smallest possible diameter and good homogeneity suitable for intravenous injection enabling direct contact of artemisinin with infected erythrocytes. Chemically and physically stable artemisinin nanoparticles were obtained with excellent entrapment efficiency. In *in vitro* experiments, the artemisinin nanoformulation was interestingly more effective than non-formulated artemisinin. In *Plasmodiumm falciparum*-infected 'humanized' mice, the nanoparticles proved to be highly effective with 96% parasitemia inhibition at 10 mg/kg/day, prolonging mean survival time without recrudescence. This nanoparticulate albumin-bound system allows the intravenous administration of artemisinin for the first time without harsh organic solvents or cosolvents with 100% bioavailability.

1. Introduction

The discovery of artemisinin (ART) by Chinese scientists in the 1970s was one of the greatest discoveries for malaria therapy towards the end of the 20th Century (Klayman, 1985). ART and its derivatives later became essential components of antimalarial treatment in artemisinin-based combination therapy (ACT). These plant-derived peroxides are unique among antimalarial drugs in killing nearly all asexual and sexual parasite forms (Ter et al., 1993; Kumar and Zheng, 1990). ART induces rapid reduction of parasite biomass estimated at about 10,000-fold per erythrocytic cycle (Cui and Su, 2009). This leads to rapid clinical and parasitological responses to treatment and life-saving benefit in severe malaria (White, 2008). Besides their strong antimalarial power, other biological activities (Ho et al., 2014) have been attributed to ART including antiviral (Efferth et al., 2008), antischistosomal (Efferth et al., 2008; Utzinger et al., 2007) and anticancer (Efferth, 2007). The latter activity was examined against many cell lines and its potency was evaluated *in vitro* and *in vivo*. Artemisinin can be compared to acetylsalicylic acid in the breadth of its pharmacological properties (Krishna et al., 2008). Another asset of ART is its apparently excellent human safety and tolerability (Price et al., 1999).

Since the initial discovery of ART, an array of oil and water-soluble derivatives has been developed by hemisynthesis. However, ART is less expensive (180–420 US $/kg) than its hemisynthetic derivatives (3500 US $/kg for dihydroartemisinin which is used to synthesize other derivatives such as artemether, artesunate) (Chaturvedi et al., 2010). In addition, ART is more chemically stable than the only water-soluble analogue artesunate (Lin et al., 1987); and displays longer elimination half-life (4.3 h) than artesunate (0.65 h) in healthy human volunteers (Benakis et al., 1997; Patil et al., 2012). No other antimalarial medicines offering

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the same level of efficacy and tolerability as artemisinins are available.

Despite being the fastest acting antimalarial drug against all erythrocytic stages of *Plasmodium* so far, ART suffers from poor solubility in water and pharmaceutical oils and extensive first-pass metabolism (Ridley, 2002; Woodrow et al., 2005; Wells et al., 2009). These physicochemical and biopharmaceutical issues limit the therapeutic potential of artemisinins to a great extent (Vennerstrom et al., 2004).

Commercially, ART is available as oral and rectal dosage forms and no intravenous (iv) formulation of ART has been available up till now. This explains the lack of absolute bioavailability studies of this molecule. Due to its poor solubility, erratic absorption, poor oral 'relative' bioavailability, extensive hepatic metabolism and autoinductive effect on cytochrome P450 enzymes, oral administration of ART is not the route of choice to achieve maximum efficacy (Woodrow et al., 2005; Gordi et al., 2002). This diminished efficacy of ART after oral administration can aggravate the problem of resistance through incomplete parasite clearance. In addition, rectal 'relative' bioavailability of ART is poor and the interindividual variability is higher after rectal administration than after an oral dosage (Karunajeewa et al., 2007; Koopmans et al., 1999). Alternative dosage forms of ART, more suitable for parenteral delivery, are therefore sought. Such a formulation can greatly enhance ART efficacy and clinical outcome, particularly in severe and cerebral malaria. These medical emergencies necessitate rapid achievement of therapeutic concentrations of the drug in the blood (Whitty and Sanderson, 1999). Therefore, the parenteral administration of a fast-acting treatment is a priority. The intravenous route (iv) ensures 100% bioavailability (Mosqueira et al., 2004) with rapid onset of action. Moreover, *iv* administration avoids the first-pass effect and minimizes the autoinduction of CYT P450 metabolizing enzymes (Cui and Su, 2009; Gordi et al., 2002).

Nanoparticles have been used recently to improve drug dissolution rate, saturation solubility, bioavailability and hence efficacy (Muller and Keck, 2004). Moreover, drug nanoformulations can achieve sustained effects which eliminates fluctuations in drug plasma concentrations and consequently avoids resistance selection due to subtherapeutic concentrations and compensates the short half-life of drugs, one of the main reasons for the high recrudescence rate (>25%) encountered with short courses of ART treatment (Cui and Su, 2009); and decrease tissue uptake and therefore toxicity (Mosqueira et al., 2004).

Various attempts at artemisinin formulation have been made. For example, Payghan et al. described a process which leads to the production of microspheres and not of nanoparticles with sizes between 595 and 420 microns (Payghan and Bhat, 2008). These sizes are enormous and cannot be injected intravenously. On the other hand the process included the addition of various chemicals (polyethylene glycol, paraffin) with only 2% albumin.

Albumin has also been used to prepare nanoparticles of primaquine. However in this work, the albumin was denatured by high temperature treatment (175–185 °C) with poor entrapment efficiency (<9%) along with low yield (20%) (Labhasetwar and Dorle, 1990). The particles are not recognized by the red blood cells and therefore the method is not suitable for preparing nanoparticles for intravenous injection.

The use of human serum albumin (HSA) to prepare albumin-bound nanoparticles of antimalarial drug is a smart concept due to its ready availability, biodegradability and lack of toxicity or immunogenicity. HSA has surface-active properties that can improve drug wettability and bioavailability and decrease particle aggregation. HSA can target malaria-infected erythrocytes as it is selectively taken up and degraded by parasitized RBCs (El Tahir et al., 2003; Duranton et al., 2008). HSA is the only adjunctive therapy that was associated with a reduction in mortality in severe and cerebral malaria (John et al., 2010). All these factors make human albumin an ideal candidate for antimalarial drug delivery. In addition, albumin-bound nanoparticles (NPs) offer organic solvent-free formulation (Elzoghby et al., 2012a).

We have recently reported the use of HSA to prepare albumin-bound nanoparticles of new antimalarial lead molecules belonging to the indolone-*N*-oxide family (Ibrahim et al., 2014). This led to very interesting antimalarial activities comparable to those of chloroquine and artesunate and even better in terms of prolonging survival time and inhibiting recrudescence. These results enabled further progress in drug development stages in this family.

The objective of this work was to produce a human serum albumin-bound, water-soluble, nanoparticulate formulation of ART for *iv* injection to overcome the physicochemical and biopharmaceutical problems associated with this drug. The HSA-bound artemisinin (ART/HSA) nanoparticles were prepared by a combination of precipitation and high pressure homogenization and characterized by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and HPLC-MS. Biological evaluation has been undertaken *in vitro* on cellular and *in vivo* on murine models.

2. Experimental

2.1. Materials

Artemisinin (98% purity, MW = 282.34 g mol^{-1}), HSA (essentially fatty acid free), chloroquine, sodium artesunate, formic acid and the organic solvents ethanol, acetone, methanol, acetonitrile and chloroform (all of HPLC grade) were purchased from Sigma–Aldrich (St. Quentin, France). RPMI 1640 medium containing L-glutamine, 25 mM HEPES (Biowest, cat n° L0495-500, www.biowet.net). [^{3}H]-Hypoxanthine, glass-fiber filter paper (Ref. 1450-421), plastic sample bag (Ref. 1450-432) and scintillation liquid (Ref. 1205-440) were purchased from PerkinElmer (Courtaboeuf, France). CO_2 gas were purchased from Air liquide Santé (Paris, France). Human RBCs (group O, Rhesus positive or negative) and human serum (group AB, Rhesus positive) were obtained from Etablissement Français du Sang (EFS, Toulouse, 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 improved by sonication in an ultrasonic bath (Elma®, Germany). Centrifugation used an Eppendorf AG® 5804R system (Hamburg, Germany)

2.2. Preparation of albumin-bound artemisinin nanoparticles

Thirty mg of ART were dissolved in 9 mL ethanol (3.3 mg/mL), the dissolution was enhanced in an ultra-sonic bath. HSA (300 mg) was dissolved in 60 mL Milli-Q water in a suitable flask. The ART solution in ethanol was added drop-wise at 1 mL/min to HSA solution under magnetic stirring. This crude suspension was subjected to evaporation under reduced pressure at 36 °C for 30 min to eliminate the ethanol. After complete evaporation of the ethanol, the crude suspension was homogenized under 25,000 psi using an Emulsiflex-C3 high-pressure homogenizer, a piston-gap homogenizer (SODEXIM S.A.S., France). A substantial increase in temperature was observed during homogenization. To avoid any deleterious effects of temperature on the suspension and to ensure that the recycled suspension was sufficiently cold, a heat exchanger was utilised through the homogenizer valve. Particle size and polydispersity index (PI) were monitored after 10, 15, 20, 25, 35 and 40 cycles. Fifty mL of the obtained nanosuspension were frozen at −80 °C for 24 h or at −195.3 °C for 90 min using liquid nitrogen prior to lyophilization (48 h at −55 °C, 6 μm Hg, Labconco