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Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application

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ABSTRACT

NLC topical formulation as an alternative to oral and parenteral (IM) delivery of artemether (ART), a poorly water-soluble drug was designed. A Phospholipon 85G-modified Gelucire 43/01 based NLC formulation containing 75% Transcutol was chosen from DSC studies and loaded with gradient concentration of ART (100–750 mg). ART-loaded NLCs were stable (−22 to −40 mV), polydispersed (0.4–0.7) with d90 size distribution range of 247–530 nm without microparticles up to one month of storage. The encapsulation efficiency (EE%) for ART in the NLC was concentration independent as 250 mg of ART loading achieved ~61%. DSC confirmed molecular dispersion of ART due to low matrix crystallinity (0.028 J/g). *Ex vivo* study showed detectable ART amounts after 20 h which gradually increased over 48 h achieving ~26% cumulative amount permeated irrespective of the applied dose. This proves that ART permeates excised human epidermis, where the current formulation served as a reservoir to gradually control drug release over an extended period of time. Full thickness skin study therefore may confirm if this is a positive signal to hope for a topical delivery system of ART.

1. Introduction

Malaria is caused by five species of parasites of the genus *Plasmodium* that affect humans (*P. falciparum*,*P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*). Malaria due to *P. falciparum* is the most deadly form and it predominates in Africa; *P. vivax* is less dangerous but more widespread, and the other three species are found much less frequently (WHO, 2013). Malaria parasites are transmitted to humans by the bite of infected female mosquitoes of more than 30 anopheline species. Globally, an estimated number of 3.4 billion people were at risk of malaria in 2013, with populations living in sub-Saharan Africa having the highest risk of acquiring malaria. Approximately 80% of cases and 90% of deaths were estimated to occur in the WHO African Region, with children under five years of age and pregnant women most severely affected (WHO, 2013; Murray et al., 2012).

Malaria is an entirely preventable and treatable disease, provided the currently recommended interventions are properly implemented (WHO, 2013). These include (i) vector control through the use of insecticide-treated nets (ITNs), indoor residual spraying (IRS) and, in some specific settings, larval control, (ii) chemoprevention for the most vulnerable populations, particularly pregnant women and infants (Dellicour et al., 2010; Cairns et al., 2012; van Eijk, 2011; Desai et al., 2007), (iii) confirmation of malaria diagnosis through microscopy or rapid diagnostic tests (RDTs) for every suspected case, and (iv) timely treatment with appropriate antimalarial medicines (according to the parasite species and any documented drug resistance) (WHO, 2013).

Antimalarial drug resistance is a major public health problem which hinders the control of malaria even at attempting some multistage, multivalented vaccines (Yang et al., 2009; Nnamani et al., 2011). Resistance is occurring as a consequence of several factors, including poor treatment practices, inadequate patient adherence to prescribed antimalarial regimens, widespread availability of artemisinin-based monotherapies, and substandard forms of the drug (Thiam et al., 2011; Wilson, 2011). In recent years, parasite resistance to artemisinins – the key compounds in artemisinin combination therapy (ACTs) – has been detected in four countries of the Greater Mekong subregion: Cambodia,

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Myanmar, Thailand, and Vietnam in agreement with the prediction of the World Health Organization (WHO) 2010 (Desai et al., 2007). Most countries where malaria is endemic have adopted the WHO recommendation of ACT for fast and reliable malaria treatment (WHO, 2006, 2010, 2013). The five ACTs currently recommended for use by WHO are artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, artesunate plus sulfadoxine-pyrimethamine, and dihydroartemisinin plus piperaquine. The choice of the ACT is based on the therapeutic efficacy of the combination in the country or area of intended use.

Artemisinin and its derivatives should not be used as monotherapies for the treatment of uncomplicated malaria, due to poor adherence to the required 7-day course of treatment which results in partial clearance of malaria parasites hence, promoting resistance to this critically important class of antimalarials (WHO, 1998, 2013). Artemisinin (Qinghaosu) is a sesquiterpene 1,2,4-trioxane (sesquiterpene lactone endoperoxide) isolated from the Chinese medicinal herb qinghao (*Artemisia annua* L.). It has been shown to be an effective antimalarial against chloroquine-resistant strains of *P. falciparum* (Klayman, 1985). This compound and its derivatives, such as artemether (ART), dihydroartemisinin, arteether, and artesunate, are effective against both chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum*, as well as against cerebral malaria (Klayman, 1985). Artemether, a potent rapidly-acting schizonticide is practically insoluble in water. It belongs to BCS class II and possesses oral bioavailability of ~45% (Mandawgade et al., 2008). The generally recommended oral and parenteral administration, once a day for at least 5 days seems reasonable in view of clinical efficacy. Yet the available marketed dosage forms are tablet, capsule and injections. While the parenteral oily formulation leads to pain on injection plus poor patient compliance, the oral formulations are rapidly but incompletely absorbed, limiting its use in malaria (Mandawgade et al., 2008). Still it is widely absorbed (GIT and kidney), distributed, and rapidly metabolized and cleared from the body with contra-indication in those with severe liver and kidney diseases, haematopathy (e.g., leucopenia or thrombocytopenia) porphyria. It interacts with drugs that increase the QT interval and has numerous adverse effects such as nausea, vomiting, skin eruption, elevated SGPT, and SGOT due to large doses (100 mg b.d). Pharmacokinetics of ART suggests that its clinical efficacy is dependent on the formulation (Karbwang et al., 1997; Bunnag et al., 1991). However, in addition to urgent need for new and effective anti-malarial agents, there is also a crucial need to utilize the existing drugs through the concept of novel drug delivery systems with the intention of reducing the dose-induced side effects, while achieving enhanced aqueous solubility, active targeting of diseased tissues, increased bioavailability and above all, patient-friendly dosage regimens to enhance compliance and reduce resistance due to non-compliance. As a result, the skin has been chosen as a route of application to assess the penetration of ART into the living epidermis as a positive signal to hope for transdermal systemic delivery into the bloodstream. This way, the longer acting antimalaria combination which requires shorter dosing intervals will remain orally active while the artemisinin component (ART) will be conveniently applied at once to the skin to synchronize the effect of ACT according to the WHO recommendation. This approach will improve patient compliance, since, the extreme nausea–vomiting tendency of ART in the ACT would be taken care of at once.

In view of this, nanostructured lipid carrier (NLC) appear to be an attractive approach for the delivery of highly lipophilic drugs such as ART as NLCs have advantages over all other colloidal systems – SLNs, SLMs, liposomes, nanoemulsions, and microemulsions (Mandawgade et al., 2008; Karbwang et al., 1997; Bunnag et al., 1991; Varshosaz et al., 2010, 2012a; Kasongo et al., 2011a). This is because the majority of drugs have higher solubility in liquid lipids (oils) rather than solid lipids. The purpose of an NLC formulation is to produce particles in which the oil is incorporated into the core of the solid lipid and the drug is solubilized in the oily core. This should result in a higher loading capacity, encapsulation efficiency, and controlled drug release as the drug dissolves in the oil and simultaneously encapsulates in the solid lipid; which should also lead to slower polymorphic transition and lower crystallinity index (higher stability) (Kasongo et al., 2011a; Varshosaz et al., 2012b,b; Teeranachaideekul et al., 2007a,b). The drug of study, ART is photolabile and short-acting, so the major criteria were to find a protective carrier with modified release property as well as good consistency (thickner). Here, Gelucire® 43/01 which is a semi-solid block hard fat (HLB 1) that protects APIs (e.g., ART) sensitive to oxidation, humidity, and light was used. Gelucire® 43/01 is also a high melting point lipid for modified release dosage forms, in addition to being a consistency agent (thickner) for topical formulations. Combination of lipophilic and hydrophilic surfactants yields better stabilization of dispersed systems. As a result, Gelucire® 43/01 was structured with a phospholipid, Phospholipon® 85G (P85G 15%), whereas the liquid lipid (Transcutol® HP) formed 75% of the entire matrix. High oil content of NLC has been associated with less crystallinity (Attama et al., 2006). Artemether-loaded NLCs were prepared and evaluated for *in vitro* and *ex vivo* performances.

2. Materials and methods

2.1. Materials

Artemether was a gift from Ipca Laboratories Ltd. India. Gelucire® 43/01 pellets (a mixture of mono, di, and triglyceride with polyethylene glycol esters of fatty acids was used as a consistency agent, sustained release matrix, and protective carrier for the photolabile drug ART), Compritol® 888 pellets and Transcutol® P (liquid highly purified diethylene glycol monoethyl ether was used as solvent for the poorly soluble ART) were kind gifts from Gattefossé, France. Phospholipon® 85G (lecithin fraction enriched with phosphotidylcholine greater or equal to 85% was used as a co-emulsifier, emollient and moisturizer as well as to increase ART skin penetration) was a gift from Lipoid GmbH, Germany. Polysorbates 80 and 20, Macrogol 4000 (surfactants) and sorbitol were obtained from Sigma–Aldrich, USA. Pluronic F68 (BASF, Germany) and Sorbitan monostearate (Span 60, Merck, Germany) were also used. Goat fat (*Capra hircus*) was from a batch prepared in the Department of Pharmaceutics, University of Nigeria, Nsukka (Nigeria). Bidistilled water was used throughout the study.

2.2. Screening of starting materials

2.2.1. Selection of lipids

First of all, thermal properties of all bulk lipids were ascertained by differential scanning calorimetry (DSC Q100 TA Instrument, Germany). Binary lipid mixtures (9:1, 8:2, and 7:3) were obtained from each solid lipid (goat fat, Gelucire 43/01 and Compritol 888 pellet) with the liquid lipid (Transcutol P) respectively at 90 °C for 2 h. The matrices were cooled for 48 h at room temperature for full recrystallization and afterwards re-investigated by DSC. Secondly, the solid lipids were also mixed at 2:1, 1:1, and 1:2 combinations to modify the crystal properties of each individual lipid. Thirdly, ternary systems were obtained from a combination of two solid lipids with the oil at ratios of 2:1, 1:1, and 1:2. All matrices were studied by DSC and the mixture that showed the least enthalpy values was selected for further investigation.