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Nanostructured lipid carriers of artemether–lumefantrine combination for intravenous therapy of cerebral malaria

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ABSTRACT

Patients with cerebral malaria (CM) are unable to take oral medication due to impaired consciousness and vomiting thus necessitating parenteral therapy. Quinine, artemether, and artesunate which are currently used for parenteral malaria therapy have their own drawbacks. The World Health Organization (WHO) has now banned monotherapy and recommends artemisinin-based combination therapy for malaria treatment. However, presently there is no intravenous formulation available for combination therapy of malaria. Artemether–Lumefantrine (ARM–LFN) is a WHO approved combination for oral malaria therapy. However, the low aqueous solubility of ARM and LFN hinders their intravenous delivery. The objective of this study was to formulate ARM–LFN nanostructured lipid carriers (NLC) for intravenous therapy of CM. ARM–LFN NLC were prepared by microemulsion template technique and characterized for size, drug content, entrapment efficiency, drug release, crystallinity, morphology, amenability to autoclaving, compatibility with infusion fluids, stability, antimalarial efficacy in mice, and toxicity in rats. The ARM–LFN NLC showed sustained drug release, amenability to autoclaving, compatibility with infusion fluids, good stability, complete parasite clearance and reversal of CM symptoms with 100% survival in *Plasmodium berghei*-infected mice, and safety in rats. The biocompatible ARM–LFN NLC fabricated by an industrially feasible technique offer a promising solution for intravenous therapy of CM.

1. Introduction

Malaria is a catastrophic parasitic infection as exemplified by the large annual mortality of nearly 0.44 million, largely due to severe malaria such as cerebral malaria (CM) (World Malaria Report, 2015). The causative agent of the most severe form of malaria, namely *Plasmodium falciparum*, has developed resistance to most of the commonly used antimalarial agents such as chloroquine, sulphadoxine–pyrimethamine, amodiaquine, quinine, mefloquine, posing a tough challenge to its elimination (Padmanaban et al., 2012). The huge antimalarial market consists largely of poor people, making it unattractive to pharmaceutical companies and hence their investment in antimalarial research is diminishing (Craft, 2008). This phenomenon of increasing resistance to existing drugs coupled with the scarcity of new drugs/drug combinations approved in recent times, places the onus of malaria control largely on smart utilization of existing antimalarials through the design of nanotechnology based drug delivery systems and combination therapy. Nanotechnology based drug delivery systems can help to overcome the drawbacks of antimalarial drugs such as poor solubility (Zhang et al., 2013), toxicity (Lim et al., 2012), poor intracellular uptake (Abed and Couvreur, 2014), and short half-life (Jaiswal et al., 2016).

The oral route is the most preferred route of drug administration. However, patients with severe malaria, in particular CM, are unable to take oral medication due to impaired consciousness often accompanied by nausea or vomiting thus necessitating parenteral therapy (Schumacher and Spinelli, 2012). CM is the most severe neurological complication of the disease observed in *P. falciparum* infection and is characterized by unarousable coma (Pradhan and Ghosh, 2013). It is the foremost cause of mortality in children under the age of five years in malaria-endemic regions (Waknine-Grinberg et al., 2013). Around 7% of *P. falciparum*-infected patients develop CM (Golenser et al., 2006).

Drugs such as quinine, artemether (ARM), and artesunate (ARS) which are currently used for parenteral therapy of severe malaria

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and CM have their own drawbacks. Quinine cannot be given by rapid intravenous injection as it can cause hypotension and cardiac arrest; it has to be given by rate controlled intravenous infusion or intramuscular injection. Intramuscular injections of quinine dihydrochloride are acidic and are painful. Quinine also causes hypoglycemia and the patients have to be monitored for glucose levels (Guidelines for the treatment of malaria, 2015). Intramuscular injection of ARM is painful and shows slow and erratic absorption (Joshi et al., 2008). ARS is available as an anhydrous powder of artesunic acid which needs to be dissolved using 5% sodium bicarbonate solution; however, the product froths and cakes on addition of the dissolution medium (Ellis et al., 2010; Guidelines for the treatment of malaria, 2015). This solution is then reconstituted with 5% dextrose and needs to be freshly prepared each time because of poor stability of the aqueous solution at neutral pH (Guidelines for the treatment of malaria, 2015). ARS also has a very short half-life of less than 15 min (Morris et al., 2011). The World Health Organization (WHO) has now banned monotherapy and recommends artemisinin-based combination therapy (ACT) for malaria treatment (Guidelines for the treatment of malaria, 2015). However, presently there is no intravenous formulation available for combination therapy of malaria. Thus there is an urgent need to develop an aqueous based formulation of ACT for intravenous administration in severe malaria and CM patients.

Artemether–lumefantrine (ARM–LFN) is a WHO approved fixed-dose oral combination containing 20 mg of ARM and 120 mg of LFN, recommended for the treatment of chloroquine-resistant *P. falciparum* malaria (Ojurongbe et al., 2013). ARM has a quick onset of action; however, it is also quickly eliminated from the plasma. LFN on the other hand has a slower onset of action and longer elimination half-life. Both the drugs have independent modes of action (Patil et al., 2013). The rationale of this combination is that ARM initially provides quick resolution of symptoms by bringing down the bulk of the parasite load, and LFN then eliminates any remaining parasites. This ensures that malaria parasites are never exposed to only ARM and helps to tackle the phenomenon of resistance. Even if the parasites are exposed to LFN alone, the probability of developing resistance simultaneously to both the drugs used in the combination is very low. ARM–LFN also decreases gametocyte carriage and hence curbs parasite transmission (Patil et al., 2013). ARM is also recommended for treatment of severe malaria (Guidelines for the treatment of malaria, 2015). ARM has been shown to cause a significant reduction in accumulation of leucocytes in brain in murine model of CM (Clemmer et al., 2011). However, low aqueous solubility of both ARM and LFN hinders their intravenous delivery (Ma et al., 2014).

Nanostructured lipid carriers (NLC) have a plethora of benefits such as simple process of manufacture, biocompatibility, enhanced drug solubility, and controlled drug release. Moreover, their ability to be selectively targeted to reservoirs of infection results in substantial dose reduction and decreased side effects thereby achieving better therapeutic outcomes at low costs and hence higher patient acceptance (Carbone et al., 2014; Jaiswal et al., 2016). Lipid nanocarriers have been explored for parenteral delivery of antimalarial drugs such as ARM (Raina et al., 2013), curcuminoids (Aditya et al., 2012), quinine (Gupta et al., 2007), and primaquine (Dierling and Cui, 2005).

Previous studies using ARM NLC developed for intravenous delivery revealed good antimalarial activity in the *Plasmodium berghei*-infected murine malaria model (Joshi et al., 2008; Patil et al., 2012). The drug-free NLC were selectively and rapidly taken up by the *Plasmodium*-infected red blood cells (RBC) (Jain et al., 2014). The NLC disrupted the tubulovesicular network (TVN) responsible for nutrient uptake by the parasite (Jain et al., 2014). The NLC also led to enhanced clearance of infected RBC by macrophages (Jain et al., 2014). Furthermore, the NLC restored the lost flexibility of the infected RBC (Jain et al., 2014). A decreased flexibility of the infected RBCs observed during falciparum malaria is thought to be responsible for the clogging of blood vessels, and precipitation of coma in CM (Dondorp et al., 2004; Pradhan and Ghosh, 2013). Hence the objective of the present study was to adapt these previously developed NLC for intravenous delivery of ARM–LFN combination, which could be used for the treatment of severe malaria and CM.

2. Materials and methods

2.1. Materials

ARM, LFN, and ARS were obtained as gift samples from Ipca Laboratories Ltd., Mumbai, India. Glyceryl dilaurate was obtained as a gift sample from Ashland Specialty Ingredients, New Jersey, USA. Capmul MCM (medium chain mono- and di-glycerides) was obtained as a gift sample from Abitec Corporation, Janesville, USA. Oleic acid, Tween 80, potassium dihydrogen orthophosphate, sodium chloride, potassium chloride, sodium bicarbonate, and disodium hydrogen orthophosphate were purchased from S.D. Fine Chemicals, Mumbai, India. Solutol HS 15 was obtained as a gift sample from BASF India Ltd., Mumbai, India. All the excipients and reagents were used as received.

2.2. Formulation of ARM–LFN NLC

ARM–LFN NLC were fabricated by the method reported by Joshi et al. (2008). ARM was solubilised in the molten mixture of glyceryl dilaurate and capmul MCM. LFN solubilised in oleic acid was added to this mixture; Tween 80 and Solutol HS 15 were then added and mixed by gentle heating at 60 °C to form a NLC preconcentrate. This preconcentrate (liquid) could then be diluted with reconstitution fluid at the time of administration.

2.3. Characterization of ARM–LFN NLC

2.3.1. Determination of globule size, polydispersity index (PDI), and zeta potential

The average globule size, size distribution (PDI), and zeta potential of ARM–LFN NLC were measured in triplicate after dilution of NLC preconcentrate with sterile water for injection (SWFI). Measurements were conducted at 25 °C using Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Zeta potential was measured on the basis of electrophoretic mobility under an electric field, as an average of 30 measurements.

2.3.2. Estimation of drug content and entrapment efficiency (EE)

Drug content of ARM–LFN NLC preconcentrate was estimated using a validated High Performance Liquid Chromatography (HPLC) method. ARM–LFN NLC preconcentrate (500 mg) was diluted to 50 ml with acetonitrile. This solution was then suitably diluted with mobile phase for estimation. For entrapment efficiency estimation, ARM–LFN NLC preconcentrate (2000 mg) was diluted to 10 ml with SWFI. This solution (1 ml) was subjected to ultracentrifugation (Optima™ MAX–XP Ultracentrifuge, Beckman Coulter, USA) at 100,000 rpm for 1 h. The supernatant was then analysed by HPLC. Entrapment efficiency was calculated by subtracting the amount of drug in supernatant from the total amount of drug added to the solution before ultracentrifugation.

2.3.3. In vitro *release studies*

In vitro release of ARM and LFN from ARM–LFN NLC was assessed in phosphate buffered saline (PBS) pH 7.4. The NLC dispersion equivalent to 20 mg ARM and 120 mg LFN was