European Journal of Pharmaceutical Sciences 40 (2010) 448–455

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Arthemeter-loaded lipid nanoparticles produced by modified thin-film hydration: Pharmacokinetics, toxicological and *in vivo* anti-malarial activity

N.P. Aditya[a], S. Patankar[b], B. Madhusudhan[a,*], R.S.R. Murthy[c], E.B. Souto[d,e,1]

[a] *Department of Biochemistry, P.G Center, Kuvempu University, Davangere 577002, Karnataka, India*
[b] *Department of Biological Sciences & Bioengineering, Indian Institute of Technology, Bombay, Powai, Mumbai 470076, India*
[c] *Department of Pharmaceutics, Indo Soviet Friendship College of Pharmacy, Moga, India*
[d] *Institute of Biotechnology and Bioengineering, Centre of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (IBB/CGB-UTAD), P.O. Box 1013, 5000-801 Vila Real, Portugal*
[e] *Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, P-4200-150 Porto, Portugal*

ARTICLE INFO

Article history:
Received 18 March 2010
Accepted 9 May 2010
Available online 20 May 2010

Keywords:
Artemether
Malaria
Lipid nanoparticles
Hepatotoxicity
Nephrotoxicity
Plasmodium berghei
Parasitemia

ABSTRACT

Artemether-loaded lipid nanoparticles (ARM-LNP) composed of 5% (w/v) lipid mass were produced by a modified thin-film hydration method using glyceryl trimyristate (solid lipid) and soybean oil (as liquid lipid in a concentration ranging from 0 to 45% (w/v) with respect to the total lipid mass). The particles were loaded with 10% of the anti-malarial ARM and surface-tailored with a combination of non-ionic, cationic or anionic surfactants. ARM-LNP were further characterized for their mean particle size, zeta potential and encapsulation efficiency, reporting optimized values below 120 nm (PI < 0.250), −38 mV and 97% (w/w), respectively. ARM-LNP composed of 45% soybean oil depicted a spherical-like shape by transmission electron microscopy and a biphasic release profile in phosphate buffer. Haemolytic activity was within the acceptable range (7%) revealing low toxicity risk of LNP for parenteral delivery of ARM. Biocompatibility was confirmed by hepato- and nephrotoxicity analyses. Histopathological analysis showed no significant histological changes in liver and kidney tissues in adult Swiss Albino mice treated with the selected formulations. *In vivo* anti-malarial activity of ARM was enhanced when formulated as LNP, in comparison to a conventional plain drug solution and to a marketed formulation which are currently in use to treat malaria patients.

1. Introduction

Artemisinin and its derivatives are among the most potent anti-malarial drugs, being active at nanomolar concentration in both chloroquine-sensitive and -resistant *Plasmodium falciparum* strains. Artemisinin has been therefore included in the WHO list of essential drugs for the treatment of severe multi-resistant malaria (WHO, 1986; Karbwang et al., 1997; Klayman, 1985).

The artemisinin derivative artemether (ARM, Fig. 1) contains sesquiterpene lactone rings with an endoperoxide bridge that is cleaved by an iron-dependent mechanism, playing a crucial role in the treatment of multi-resistant malaria parasite. It. Artemether has been found to inhibit haemozoin formation as well as haemoglobin degradation, due to the presence of the haem group that is a potent inhibitor of cysteine protease (Klayman, 1985). However, this drug holds some important shortcomings, including (White et al., 1999): (i) short half-life usually between 3 h and 5 h; (ii) poor aqueous solubility, and thus low oral bioavailability (~40%); (iii) risk of degradation in acidic conditions; and (iv) associated risk of toxicity (Akinlolu and Shokunbi, 2010). Intramuscular (i.m.) injections currently available in the market are associated with low patient compliance and inconsistent assimilation (Hien et al., 2004). In addition, i.m. administration is not suited to deliver the drug to treat cerebral malaria, or when speedy suppression of the parasite is required (Li et al., 1998). Developing novel approaches to administer these derivatives by some alternative parenteral route would be valuable in overcoming their therapeutic limitations.

The scientific research devoted to parasitic diseases faces a lack of financial inputs towards the development and discovery of new drug entities. Suitable alternatives to overcome such limitations would be the improvement of *in vivo* performance of well known anti-malarial drugs. To pursue such attempt, some researchers worldwide are involved in developing advanced drug delivery systems to enhance the anti-malarial activity of artemether (Wang et al., 2007, 2006; Mu et al., 2008). Therefore, a promising strategy to tackle novel approaches would be developing lipid nanoparticles

* Corresponding author. Tel.: +91 9880548239; fax: +91 8192 208135.
E-mail addresses: basavaraj_madhu@hotmail.com (B. Madhusudhan), eliana@ufp.edu.pt (E.B. Souto).
[1] Tel.: +351 22 507 4630; fax: +351 22 550 4637.

0928-0987/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejps.2010.05.007

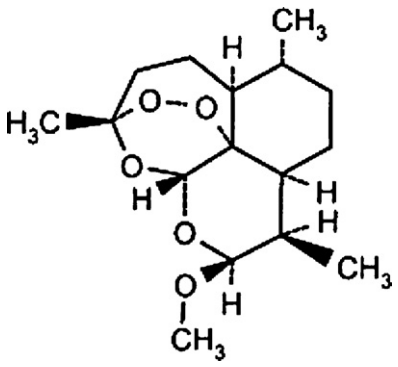

Fig. 1. Chemical structure of artemether (MW 298 kDa).

(LNP) consisting of a solid matrix (melting point > 40 °C) prepared by blending solid lipid (>70%, w/w) with increasing ratio of liquid lipid (i.e., oil up to 30%, w/w) (Zhang et al., 2010). The present work reports the production of LNP composed of Trimyrstin (triglyceride of C_{14}) and soybean oil, which have been loaded with ARM to boost its anti-malarial activity.

2. Materials and methods

2.1. Chemicals

The solid lipid Dynasan 114 (Trimyristin, glyceryl trimyristate), was obtained from Sasol (Witten, Germany) and received as a kind gift from Prof. R. H. Müller, Berlin University, Germany. Artemether was obtained from M/S Themis Medicare Ltd. (Gujrat, India) and received as kind gift from Prof. G. Padmanabhan, IISc, Bangalore, India. Soybean oil was provided by Kamani Oil Industries Ltd. (Mumbai, India). Tween 80 (polyoxyethylene sorbitan monooleate), lecithin soya, methanol, chloroform and sodium deoxycholate were purchased from HI MEDIA (Mumbai, India). Sterylamine was obtained from Sigma–Aldrich (Bangalore, India), sucrose from s.d. Fine Chemicals (Mumbai, India), and Poloxamer 188 (polyoxyethylene-polyoxypropylene copolymer) from BASF (Rhineland-Palatinate, Germany). Field stain A (buffered solution of azure dye) and Field stain B (buffered solution of eosin) were purchased from Alpha Chemika (Mumbai, India). Marketed drug formulation containing 1.25 mg/ml of arthemeter was obtained from Emal M/S Themis Medicare Ltd. (Gujrat, India).

2.2. HPLC analysis

Artemether was quantified by HPLC as described by Cesar Ida et al. (2008). HPLC assay was carried out on a Jasco AS-2057 system (Palo Alto, CA, USA), based on a quaternary pump, autosampler, Diode Array Detector (DAD) and HP Chem Station software. The column HiQ SIL C18 (250 mm × 4.6 mm i.d.; 5 μm particle size) from Waters (Milford, MA, USA), was maintained at 30 °C. UV spectra from 190 nm to 400 nm were online recorded for peak identification. The injection volume was 20 μl. An isocratic mobile phase containing acetonitrile and 0.05% trifluoroacetic acid (80:20, v/v) was used at a flow rate of 1.0 ml/min.

2.3. Preparation of LNP formulations

LNP containing the anti-malarial arthemeter (ARM) were prepared by the modified thin-film hydration method as described by Venkateswarlu and Manjunath (2004). Briefly, the lipid phase, consisting of 5% (w/w) solid lipid (Trimyristin) and increasing concentrations (0%, 15%, 30% and 45%, w/w) of liquid lipid (soybean oil), 10% (w/w) of drug, and 0.75% (w/w) of soybean phosphatidyl choline (PC) were dissolved in a mixture of chloroform and methanol (2:1). The solvent was evaporated from the mixture under vacuum using a Buchi rotavapour (400 mbar, 65 °C). Nitrogen was blown onto the lipid layer to remove vapour traces of organic solvents if any. An aqueous phase was prepared by dissolving 1.5% Poloxamer 188, 1.5% Tween 80 and 0.75% sodium deoxycholate in double-distilled water and heated to same temperature of the molten lipid phase. This hot aqueous phase was added to the thin lipid film and hydrated for 30 min at 65 °C. A coarse hot o/w emulsion was obtained and was further submitted to an ultrasonicator (SON-1VCX130, Sonics and materials, Inc, Newton, USA) for 15 min. ARM-loaded LNP were obtained by allowing the hot nanoemulsion to cool to room temperature. Stearylamine 0.25% (w/w) was used as surface charge modifier, added to the lipid phase previously the production. The developed ARM-loaded LNP were identified as ARM-N0, ARM-N15, ARM-N30, ARM-N45 and ARM-N45s. Numbers stand for % of liquid lipid (from 0% to 45% w/w) and "s" for stearylamine. Five ARM-free (blank) LNP, coaded as BL-N0, BL-N15, BL-N30, BL-N45 and BL-N45s, were produced by the same process however without adding the anti-malarial drug to the lipid phase. Table 1 summarizes the composition of developed LNP formulations. All samples were prepared in triplicate for further analysis.

To study the effect of the processing parameters on the physicochemical properties of LNP, blank formulations were produced by varying the following conditions: (i) concentration of the total

Table 1
Composition of developed artemether-loaded and arthemeter-free (blank) LNP.

Formulation	Trimyristin	Soybean oil	Artemether	Soybean PC	Poloxamer 188	Tween 80	Sodium deoxycholate	Water ad
ARM-free LNP								
BL-N0	100	00	–	150	300	300	150	20
BL-N15	85	15	–	150	300	300	150	20
BL-N30	70	30	–	150	300	300	150	20
BL-N45	50	50	–	150	300	300	150	20
BL-N45s	50	50	–	150	300	300	150	20
ARM-loaded LNP								
ARM-N0	100	00	00	100	150	300	300	150
ARM-N15	85	15	15	300	150	300	300	150
ARM-N30	70	30	30	100	150	300	300	150
ARM-N45	50	50	50	100	150	300	300	150
ARM-N45s	50	50	50	100	150	300	300	150

BL, blank; ARM, artemether; s, stearylamine; PC, phosphatidylcholine; ad, added.