Sustained-release Liquisolid Compacts of Lumenfantrine-Artemether as Alternate-Day-Regimen for Malaria Treatment to Improve Patient Compliance

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ABSTRACT

Lumenfantrine SLNs (LUM-SLNs, 0.2, 0.5, 0.8 and 1%w/w) were formulated with Precirol-Transcutol (PT) and Tallow fat-Transcutol (TT) at 3:1 ratios using Poloxamer 188, Polysorbate 80 and Solutol HS by hot homogenization. Thermal properties, particle size/morphology, ZP, PDI, EE, interaction study and *in vitro* drug release were carried out. LUM-SLNs were directly compressed with artemether into liquisolid compacts (AL). *In-vitro* release was done in biorelevant media. Peter's 4-day curative test was done in CQ-sensitive strain of *Plasmodium berghei berghei*. Nanoparticles were formed with high % yield, stability (-24.8 to–29.2), low enthalpy and high EE (70-95 %). Liquisolid compacts sustained LUM release in SIF (84.32%, PT-SLN) better than (77.9%, SLN-TT). Non-Fickian (anomalous) diffusion and super case II transport were predominant mechanisms. Equal parasitaemia reduction was seen for all particles (~92%) superior to 86% from Coartem® and 66% commercial CQ-PO_4. No significant difference ($p<0.05$) between double (4/24 mg/kg) and single (2/12 mg/kg) strength doses of artemether-lumenfantrine (AL) compacts was seen. AL could be formulated in lower doses (4/24 mg/kg) and taken orally once-in-two days to improve patient compliance which is currently problematic with conventional forms containing 20/120 mg of AL and taken for 6 doses, in addition to erratic absorption and numerous side effects due to large doses.

Keywords: malaria; solid lipid nanoparticles; liquisolid compacts; artemether; lumenfantrine

1 INTRODUCTION

Malaria is an entirely preventable and treatable disease, provided the recommended interventions are implemented (i) vector control by use of insecticide-treated nets (ITNs), indoor residual spraying (IRS), larval control, (ii) chemoprevention for pregnant women and infants (iii) confirmation of malaria diagnosis through microscopy or rapid diagnostic tests (RDTs) for every suspected case, and (iv) timely treatment with appropriate antimalarial drug [1]. Artemisinin combination therapy (ACT) is mainstay in malaria treatment due to resistance including artemether/lumefantrine (AL), artesunate/amodiaquine, artesunate/mefloquine,artesunate/sulfadoxinepyrimethamine, and dihydroartemisinin/piperaquine [2]. Compliance to 5-7 days long treatment courses with large doses 20/120 mg and numerous side effects is a problem [2-4]. Here, we demonstrate that AL can be reformulated in small doses 4/24 mg/kg using novel nanoparticle drug delivery systems and liquisolid compacts taken alternate days (once-in-two days) to achieve clinical cure due to sustained clearance of parasite in the blood thereby improving compliance and reducing resistance due to non-compliance.

2 METHODS

2.1 Formulation of Particles

Hot homogenization technique was adopted and 15 % lipid matrix of Precirol® ATO 5/Transcutol® HP (PT) and Tallow fat/ tallow fat (TT) at 3:1 ratio each was melted at 90 ºC and loaded with LUM (0.2, 0.5, 0.8 and 1 % w/w) to which 3 %w/w Solutol® HS 15 and 1 %w/w Polysorbate 80 were added to enhance LUM lipid solubility. Aqueous phase containing 1 %w/w Poloxamer® 188 at same temperature was added to molten lipid and subjected to high shear homogenization (Ultra-Turrax) at 25,000 rpm for 15 min to produce an o/w emulsion. The emulsion was allowed to cool at room temperature. SLN containing no drug was also formulated to serve as negative control.

2.2 Determination of Percentage Yield

Done by weighing each SLN batch after formulation and percentage (%) yield calculated using Equation 1.

2.3 Particles Size and Morphology:

Mean diameter, zeta potential and polydispersity index of SLNs were measured using a zetasizer nano-ZS (Malvern Instrument, Worceshtire, UK) while transmission electron microscope (JOEL 1210, JOEL. Inc., Boston, MA, USA) was used to observe SLNs microstructures.

2.4 Crystallinity and Polymorphism

DSC was used to ascertain thermal properties of SLNs at heating rates of 10 °C/min between 35 -190 °C

under a 20 ml/min nitrogen flux with sample size of 3-5mg, using an empty standard aluminium pan as reference.

2.5 Entrapment efficiency of SLNs

Vivaspin filter tubes of 10,000 MWCO were used with 5 ml undiluted SLN formulation placed in the upper chamber and centrifuged at 10,000 rpm for 2 h at intervals. Drug content was estimated by reference to the standard Beers plot using equation 2.

2.6 FT-IR Spectroscopy

Compatibility between the pure drugs and lipid matrix was studied using a Shimadzu FTIR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) and spectra were recorded in the wavelength region of 4000 to 400 cm^{-1} with threshold of 1.303, sensitivity of 50 and resolution of 2 cm^{-1}.

2.7 Formulation of Liquisolid Compacts of AL

Optimized samples of LUM-SLNs (200 mg) were formulated into tablets using DCE of 0.5 % Colloidal silicon dioxide, 40 mg artemether, 2 mg Magnesium stearate and sufficient quantity of MCC. Tablets were evaluated for uniformity of weight and drug content, hardness, friability, disintegration time and *in vitro* dissolution rate tests.

2.8 *In Vivo* Schizontocidal Activity

Evaluation of the curative potential of formulated tablets against established *Plasmodium* infection in rats was carried out according to standard protocols [5]. On day 0, % parasitaemia and red blood cell count of the donor mice was determined by Giemsa-stained thin blood smear of the donor mice obtained by cardiac puncture and from the retro-orbital plexus, and diluted with physiological saline to give a concentration of 108 parasitized erythrocytes per ml. Some 2 × 10^7 parasitized erythrocytes (0.2 ml of 108 parasitized erythrocytes/ml) was injected intraperitoneally into each of the experimental mice. Thirty mice were all inoculated with chloroquine sensitive strain of *Plasmodium berghei berghei* (NK 65) and left untreated until the fourth day (D_4) post inoculation. The mice were weighed and randomized into six groups of five mice each. Group A-F were administered with 0.2 ml/kg of normal saline; 10 mg/kg (on day 1) and 5 mg/kg (on day 2 and 3) of chloroquine tablet; 4mg and 24 mg/kg of AL compact once daily; 4mg and 24 mg/kg of commercial AL (Coartem®); 2mg/kg and 12 mg/kg of AL compact b.d; 4mg/kg and 24 mg/kg of AL compact once daily; 2mg/kg and 12 mg/kg of AL compact b.d. On day-7, each mouse was tail-bled and a thin blood film was made on a microscope slide and stained with 10% Giemsa solution and examined microscopically to ascertain parasitaemia level. Antimalarial activity was determined by equation 3: Data was statistically analyzed.

3. RESULTS

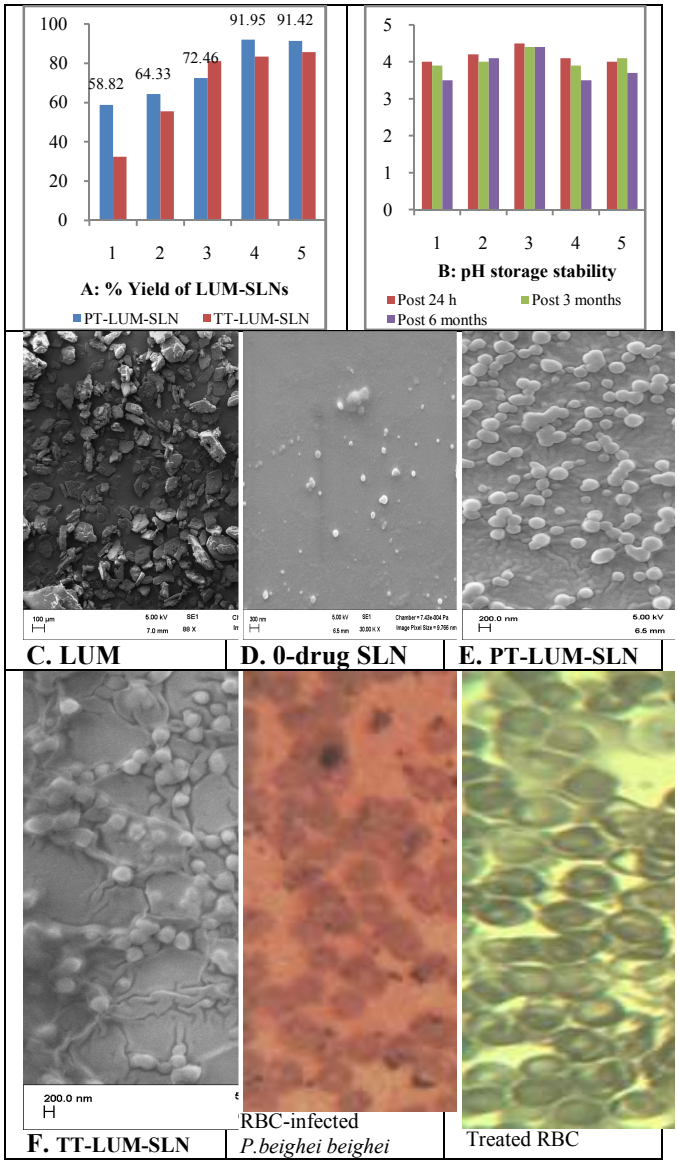

Table 1: Properties of LUM-SLNs

Batches	ZP (mV)	PDI	Z-Ave	%EE	Enthalpy (-mW/mg)
PT-SLN0	-28.9	0.58	570.3	-	37.59
PT-SLN0.8	-27.4	0.73	900	93.2	34.18
TT-SLN0	-31.6	0.48	655.9	-	28.0
TT-SLN0.5	-17.3	0.74	896.9	94.8	23.33

ZP means Zeta potential; PDI means Polydispersity index; Z-Average means Average particle size and EE means Encapsulation efficiency.

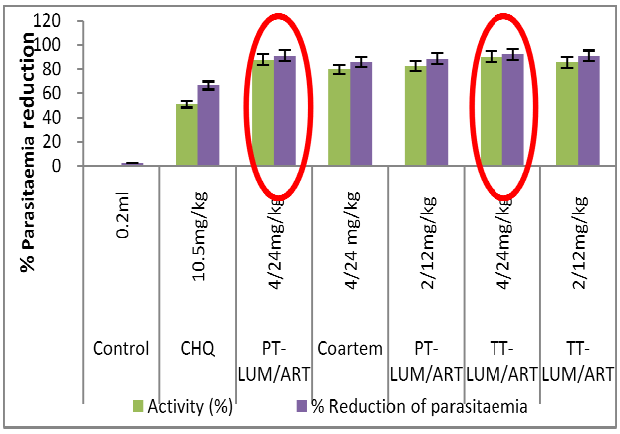

Table 2: Compact properties

Code	Wt (mg)	Th. (mm)	D (mm)	Friab (%)	Av-H (Kp)	Dt (h)
AL_1	400	4.15	11.10	1.22	1.2	3.10
AL_4	380	4.30	11.10	1.4	1.4	6.30
BL_1	395	4.10	11.10	0.51	0.5	25.30
BL_3	370	4.00	11.10	0.66	1.0	25.60

AL_1 means placebo compact while AL_4 means PT-LUM/ART0.8
BL_1 means placebo compact while BL_4 means TT-LUM/ART0.5
Wt means Weight uniformity; Th means Thickness; D means diameter; Friab means Friability; Av-H means Average height and Dt means Distingeration time.

4 DISCUSSION

Nanoparticles were polydisperse but stable over one year. LUM-SLNs had lower enthalpies than 0-SLN confirming low crystallinity and polymorphism of particles (Table 1). EE was higher (~95 %) in optimized TT-SLN containing 0.5 % LUM than in PT-SLN (93%) containing 0.8% LUM though % yield of particles was in the reverse order. Since characteristic peaks detected in pure drugs and lipid matrix were still maintained in the spectra of the different formulations, it could be concluded that no strong chemical interaction leading to formation of entirely new compounds occurred between them. LUM-Artemether compacts (AL batches) had acceptable properties (Table 2).

Equal reduction in parasitaemia was observed for AL_4 of PT batch (91%) and BL_3 of TT batch (92%), both superior to 86% from commercial sample (Coartem®) and 66% commercial chloroquine phosphate. There was a significant difference ($p<0.05$) between double strength dose (4/24 mg/kg) and single strength dose (2/12 mg/kg) of artemether-lumenfantrine (AL) in liquisolid compacts. This implies that AL can be formulated in lower doses (4/24 mg/kg) and taken orally once-in-two days to achieve ~92% parasitaemia reduction, to improve patient compliance which is currently problematic with conventional forms (85%) containing 20/120 mg of AL and taken for 6 doses; 1st and 2nd doses at 8 hourly intervals then the rest at 12 hourly intervals; in addition to erratic absorption and numerous side effects due to large doses.

It can therefore be concluded that the AL-type of antimalarial ACT could conveniently be reformulated using novel DDS to reduce dose and be taken once-in-two-days to increase patient compliance instead of the conventional b.d. regimen with high doses making for numerous side effects and non-compliance with attendeant parasite resistance.

5. EQUATIONS

$$\%\ Recovery = \frac{W1}{W2 + W3}\ X\ 100 \qquad (1)$$

$$\%EE = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} X\ 100 \quad (2)$$

$$\%\ Activity = \frac{Mean\ parasitaemia\ in\ treated\ group}{Mean\ parasitaemia\ in\ control\ group}\ X\ 100 \quad (3)$$

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