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PRELIMINARY INVESTIGATION ON THE FUNCTIONAL PROPERTIES OF A NANOLIPOSOME FOR PARENTERAL DELIVERY OF ARTEMETHER-LUMEFANTRINE

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ABSTRACT

Artemisinin have remained the stable drug of choice in the treatment of uncomplicated malaria. Unfortunately, it has poor aqueous solubility. This preliminary study seeks to investigate the functional properties of lumefantrine and artemether encapsulated in a nanoliposome for parenteral administration. Nanoliposomes encapsulating artemether and lumefantrine were formulated through the Thin-Layer Evaporation method and evaluated for particle size, polydispersity index, encapsulation efficiency, and stability. The mean particle size of the empty nanoliposomes was 120.7 nm, while that of the drug-loaded carrier was 108.7 nm. The drug-loaded liposome had a polydispersity index of 0.197, with the encapsulation efficiency of arthermether and lumefantrine being 79.4 % and 36.1 %, respectively. The liposomes showed good stability in the presence of increasing concentrations of Triton X-100. Overall, this study has shown that nanoliposomes have a great potential for possible use in the parenteral administration of artemether and lumefantrine.

Keywords: nanoliposomes, artemether, lumefantrine, stability, malaria

INTRODUCTION

Due to its ability to increase the bioavailability of drugs and reduce the toxicity associated with conventional drugs, nanotechnology have seen increased use in human medicine (Attama *et al*., 2016). Liposomes are nanosized colloidal vesicles consisting of an amphiphilic lipid bilayer that enclose a hydrophilic core (Elbayoumi and Torchilin, 2010). Liposomes are biocompatible and biodegradable, and are used as nanocarriers for the delivery of active molecules to different biological sites (Hussain *et al*., 2017). Depending on the method of preparation and purpose, their dimensions can vary from tens to hundreds of nanometer (Carugo *et al*., 2016). Long circulating liposomes can be generated by modulating their surface chemistry, lipid composition, size, and charge (Akbarzadeh *et al*., 2013). Addition of cholesterol during the preparation of the lipid carriers increases their rigidity and stability, with some reports of the liposomes being stored intact for up to 9 months at a low temperature (Nguyen *et al*., 2016). Also, addition of the hydrophilic polyethylene glycols can confer protection and stability to the liposomes (Pasut *et al*., 2016). Artemether and lumefantrine are poorly soluble drugs that require lipidic nanocarriers like liposomes, ethosomes, and solid lipid microparticles to improve their absorption and bioavailability (Thakur *et al*., 2018; Singhvi *et al*., 2018). Delivering artemether-lumefantrine as an injection would reduce the huge loss of young lives to severe malaria as well as antimalarial resistance which is not achieved with oral tablets (Hoglund *et al*., 2018). Furthermore, gastrointestinal intolerance and erratic intestinal absorption make the oral route of administration unreliable in many patients (Trampuz *et al*., 2003). This research is an attempt to characterize nanoliposomes for parenteral delivery of artemether-lumefantrine.

MATERIALS AND METHODS

Materials

Lipoid S75 fat-free soybean phospholipids with 70%n-phosphatidylcholine (Lipoid GMBH Fringenstrasse 4.D-67065 Ludwigshafen), Cholesterol (Lanolin Biochemika, Fluka), distearoyl phosphoethanolamine (DSPE) (Coatsome ME 8080 NOF Corporation, Lot No 151286 IL), Artemether (Sigma, USA), Lumefantrine (Sigma, USA), Millipore

water.

Methods

Preparation of liposomes

Conventional liposomes consisting of lipoid S75, DSPE, cholesterol, lumefantrine, and artemether in a molar ratio of 6:1:1:2:1 were prepared primarily by the Thin-Layer Evaporation method. Briefly described, the mixed lipids and drugs were solubilized in 2 mL chloroform, followed by the removal of the organic solvent to obtain a thin lipid film. All traces of the organic solvent were removed by placing the thin lipid film overnight in a dessicator. Subsequently, the lipid film was hydrated with 1 ml of phosphate buffered saline (PBS-1x) for 1h in a thermomixer (under mild stirring (37ºC, 300 rpm). During this process, freeze-thaw cycles were performed three times in liquid-nitrogen (-196ºC) and a thermomixer (37ºC), respectively. The empty liposomal vesicles or drug-loaded liposomes were sonicated (Omni Ruptor 250, Omni International Inc, Ultrasonic homogenizer) for 1 h and extruded through a stainless steel extrusion device using polycarbonate filters with 400 nm, 200 nm, and 100 nm pores.

Quantitative determination of artemether-lumefantrine by RP-HPLC

The High Performance Liquid Chromatography (HPLC) analysis was carried out on Agilent 1260 Infinity (PaloAlto, CA, USA), composed of a quaternary pump, autosampler, diode array detector (DAD), and a HP Chemstation software.

Calibration plots for artemether and lumefantrine were determined by HPLC (Agilent 1260 Infinity, Jupiter 5µ C18300 R, 250 X 4.60 mm column) for determination of encapsulation efficiency. The solvent gradient system of acetonitrile/0.05% TFA (60:40; 5:95; 60:40) was used with a UV detection wavelength of ~~at~~ 210 nm (artemether) and 335 nm (lumefantrine), a retention time of 13 min, a flow rate of 1.0 mL/min, and an injection volume of 10 µL maximum. The R^2 for lumefantrine was 0.999, while that of artemether was 0.998. For the separation of the drug not encapsulated by the liposomes and to evaluate drug entrapment efficiency, the PD-10 Gel filtration chromatography (GE Healthcare, USA) was applied. After gel filtration, the percent drug encapsulation efficiency (EE) for artemether and lumefantrine was calculated.

Physicochemical characterization of the conventional liposomes

Dynamic laser scattering analysis was carried out using a Zetasizer Nano ZS apparatus (Malvern Instruments Ltd.,

Worchestershire, United Kingdom) to evaluate the mean size and size distribution of the liposomes. The following equipment and settings were used: a laser diode (4.5 mW, 670 nm), a backscattering photon angle detector (173°), a real refractive index (1.59), a medium refractive index (1.33), and an imaginary index of zero.

Quantitative determination of phospholipids by Stewart's assay and stability studies with Triton X-100

A stock solution of lipoid S75 at a concentration of 0.1 mg/mL in chloroform was prepared and was diluted in ammonium ferrothiocyanate to prepare the calibration plot. The solutions were subjected to vortexing (Velp Scientifica, AdvancedVortex mixer) for 20 s and then centrifuged at 1000 rpm for 10 min. The lower clear layer was removed using a Pasteur pipette and the absorbance of the organic phase read at a wavelength of 485 nm (Evolution 201 UV-VIS Spectrophotometer, Thermo Scientific). This was used to calculate the molarity of S75. The empty conventional liposomes were then subjected to Triton X-100 titration and monitored using the dynamic light scattering (Zetasizer, Malvern Instruments). In details, aliquots of a surfactant solution of Triton X-100 were added into a single-use polystyrene half-microcuvettes with a pathlength of 10 mm, containing 170 µl of lipid vesicles at a lipid concentration of 3 mM. All experiments were performed at 37°C. The average size after each addition of Triton X-100 was measured after an equilibration time of 900 s. Each sample was recorded three times with 10 sub-runs of 10 s using the multimodal mode (Pasut *et al*., 2016). The data were reported as the mean of three different experiments ± standard deviation.

RESULTS

Particle size and polydispersity index

The size of the drug-loaded nanoliposomes were <130 nm and the polydispersity index < 0.2 after extrusion using the polycarbonate membranes.

Encapsulation efficiency

The encapsulation efficiency (EE) for artemether was 79.4% while the EE for lumefantrine was 36.1%. Gel filtration chromatography was used to exclude unentrapped drug. Subsequently, the membrane of the liposomes was disrupted using ethanol: liposomes at a ratio of 15:1 by vortexing and spinning in a microcentrifuge at a speed of 6000 rpm for 60 s. The disrupted liposomes were analyzed using HPLC and the concentration of the analytes was obtained. The calibration

plots of pure lumefantrine and artemether showed an $R^2 > 0.9$.

Stability studies with Triton X-100

The calculated concentration of S75 in the liposome was 24 mM. A dilution of 3 mM of the liposome was made to ascertain its stability with an increasing concentration of Triton X-100. The liposomes were disrupted with 15 µL of 10 mM of Triton X-100, giving a size of 13.1 nm. This was measured using the Dynamic Light Scattering (DLS) which measures the size of the colloidal vesicles. It was observed that at higher concentrations of Triton X-100, the size of the conventional liposomes increased until the membrane eventually disrupted at the solubilization boundary. The polydispersity index also showed a linear correlation with the surfactant concentration until destabilized.

DISCUSSION

Particle size and polydispersity index

Since the nanoliposomes were prepared for system circulation, controlling the size is important because it affects its pharmacokinetics, tissue distribution, and clearance (Danaei *et al*., 2018). Particle sizes greater than 500 nm are marked for clearance by opsonins and subsequently phagocytosed by macrophages (Onuigbo *et al*., 2012). Sonication and extrusion methods produce nanosized unilamellar vesicles with polydispersity index (PdI) less than 0.2. Such PdI values usually produce a monodisperse distribution that is appropriate for systemic circulation (Manosroi *et al*., 2010; Muzzalupo *et al*., 2008). Incorporating lumefantrine and artemether which are highly lipophilic in a liposome required a phospholipid like S75 with a large molecular weight to anchor the lipophilic drugs in its bilayers. The DSPE provided charge repulsion between vesicles which prevented aggregation and therefore stabilized the membrane. Intravenous administration of the artemether-lumefantrine entrapped within the liposomes provides a direct and expedited targeting of the merozoites which is critical in antimalarial treatment. In *P. falciparum* malaria, infected erythrocytes adhere to the endothelium of capillaries and post-capillary venules leading to obstruction of the microcirculation and localized anoxia (Paget, 2009).

Encapsulation efficiency

Lumefantrine has a high molecular weight and is highly lipophilic which prompted a large amount of S75 used in the preparation. The low encapsulation efficiency of lumefantrine was due to its adhering to the polycarbonate filters. Artemether has a

lower molecular weight and is less lipophilic than lumefantrine. It had a relatively high encapsulation in the liposomes because of easier passage through the polycarbonate filters. Encapsulation depends on the method of preparation, the nature of the drug, and phospholipids (Balakrishnan *et al*., 2009; Shahiwala and Misra, 2002).

Stability studies with Triton X-100

The liposome showed strong stability despite adding increasing concentrations of the Triton X-100. Studies have also shown that particle sizes drop abruptly at the solubilization boundary (Pasut *et al*., 2016). Stability of the liposomes is important to maintain the integrity of the liposomes and to avoid drug leakage during storage and in circulation (Verma *et al*., 2010).

CONCLUSION

The nanoliposomes using S75 as the phospholipid with low mean particle size, PdI, and good stability is a promising candidate for parenteral delivery of artemether and lumefantrine. Further investigations to determine the release kinetics and antimalarial studies in mice would need to be done.

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