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Original Research Article

Comparison of classical, stealth and super-stealth liposomes for intravenous delivery of lumefantrine: Formulation, characterization and pharmacodynamic study

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Abstract

Purpose: *To develop and compare classical liposomes (CL), stealth liposomes (SL) and super-stealth liposomes (SSL) encapsulating lumefantrine for intravenous administration.*
Method: *CL, SL or SSL were prepared by thin-layer evaporation method and evaluated for particle size, polydispersity index (PdI), encapsulation efficiency and short-term stability. Pharmacodynamic study using mice infected with Plasmodium berghei was also carried out.*
Results: *The particle sizes (nm) and PDI of the liposomes were: CL (248 ± 44.89; 0.78 ± 0.02), SL (235.8 ± 45.18; 0.39 ± 0.06) and SSL (238.2 ± 23.0; 0.24 ± 0.04). Encapsulation efficiency was highest in SSL (66 %), followed by SL (44.4 %) and then by CL (42.5 %). SSL was the most stable after 72 h of storage. In vivo, lumefantrine produced significant reduction in parasitaemia after 7 days ($p < 0.05$) by SSL (68.3 ± 8.9 %) followed by CL (55.8 ± 15.2 %) and then SL (53.4 ± 14.9 %).*
Conclusion: *SSL formulation of lumefantrine exhibits good physicochemical and pharmacodynamic potentials and should be further investigated in future studies for the treatment of malaria.*

Keywords: *Drug delivery, Classical liposomes, Stealth liposomes, Super-stealth liposomes, Lumefantrine, Malaria*

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INTRODUCTION

Malaria is a major health concern being the cause of more than one million deaths every year worldwide [1,2]. It is transmitted by Anopheles mosquitoes, the hexapod invertebrates belonging to the Culicidae family of Insecta class [3-6]. For uncomplicated malaria, World Health Organization (WHO) has recommended the oral administration of artemether-lumefantrine [7]. Artemether has a fast onset of action while lumefantrine has delayed onset but a longer duration [8]. However, artemether-lumefantrine is not available as an injection. Owing to the huge loss of lives due to severe malaria and antimalarial resistance in endemic areas, the intravenous administration of artemether-lumefantrine encapsulated in liposomes holds good potentials to increase bioavailability and efficacy, which is not achieved with the oral

tablets [9]. Liposomes are amphiphilic biocompatible and biodegradable vesicle nanocarriers, consisting of one or more concentric bilayers of natural and/or synthetic phospholipids and cholesterol enclosing hydrophilic drugs within the aqueous core or lipophilic drugs within the phospholipid bilayer [10-13]. They have different physicochemical properties; therefore, they can be used through different routes of administration [14,15]. Improving the delivery of hydrophobic lumefantrine by leveraging on the physicochemical properties of a liposome for parenteral delivery would impact on malaria control by reducing the losses encountered in severe malaria [16].

This research evaluates the preparation and characterization of liposomal formulations of lumefantrine for the treatment of malaria. We prepared stabilized stealth liposomes (super stealth liposomes), obtained by using polyethylene glycol chains with phospholipid units per polymer chain, to develop a long-lasting formulation of the antimalarial drug lumefantrine. This formulation was compared with classic and stealth liposomes of the same drug from the point of view of stability and pharmacodynamic activity in an animal model.

EXPERIMENTAL

Materials

Lipoid S75 fat-free Soybean phospholipids with 70 % phosphatidylcholine (Lipoid GMBH Fringenstrasse 4.D-67065.Ludwigshafen, Germany), Cholesterol (Lanolin Biochemika, Fluka, Sweden), distearoyl phosphoethanolamine (DSPE) (NOF Corporation, Germany), Lumefantrine (Sigma, USA), and methoxy polyethylene glycol distearoyl phosphoethanolamine $mPEG_{5000}$-$(DSPE)_2$ was synthesized in the laboratory as described.

Animals

Thirty male Wistar mice were used for the experiment. The mice were housed in clean cages and were fed on rationed mash and clean water. Two donor mice infected with chloroquine-sensitive *Plasmodium berghei* were purchased from Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria.

Synthesis of $mPEG_{5000}$-$(DSPE)_2$

Repeated steps of β-Glutamic acid derivatization and carboxyl group activation were performed starting from a linear mPEG-NHS of 5 kDa and then coupling with 2 DSPE molecules. Briefly, 1g of $mPEG_{5kDa}$-NHS (1 eq, 0.198 mmol, MW 5056 Da, 81 % activation degree) was reacted overnight with 87 mg of β-Glutamic acid (βGlu, 3 eq, 0.591 mmol, MW 147.13 Da), previously dissolved in 0.1 M borate buffer pH 8.0/ACN 3:2 v/v, at a final polymer concentration of 10 % w/v. The reaction mixture was acidified to pH 5.0 with 1M HCland after acetonitrile (ACN) removal by rotary evaporation, the product was purified by extractions with dichloromethane and precipitation in cold diethyl ether. The product was recovered by filtration and dried under vacuum overnight (0.98 g, 98 %w/w yield). The two carboxyl groups of βGlu were then activated to succinimide ester by reaction with 238 mg of *N,N′*-dicychlohexylcarbodiimide (DCC, 6 eq, 1.156 mmol, MW 206.33 Da) and 67 mg of N-Hydroxysuccinimide (NHS, 3 eq, 0.578 mmol, MW 115.09 Da) in anhydrous dichloromethane (CH_2Cl_2), at a final polymer concentration of 10 %w/v.

The degree of activation was calculated according to Snyder and Sobocinsky assay. An additional step of βGlu conjugation and NHS/DCC activation was performed, obtaining mPEG-βGlu$(βGlu)_2$-$(NHS)_4$ (0.56 g) that allowed the coupling of two DSPE molecules in the following step. Subsequently, 161 mg of DSPE (2.2 eq, 0.215 mmol, MW 748.1 Da) was dissolved in $CHCl_3$ at 45 °C by the addition of 33 µL of triethylamine (1.2 eq in excess to each carboxyl group, 0.235 mmol, MW 101.19 Da, d = 0.73 g/cm^3) and added to the solution of activated polymer, at a final polymer concentration of 10 % w/v. The reaction was stirred for 3 h (reflux) and monitored by TLC using as eluent $CHCl_3$:MeOH 8:2 v/v +0.2% H_2O and I_2 vapors for PEG visualization or 0.2 %w/v ninhydrin in EtOH solution for DSPE visualization. The product was recovered by lyophilization and characterized by ^{1}HNMR spectroscopy.

Preparation of liposomes

CL, SL or SSL was prepared by thin-layer evaporation method. Briefly, after solubilization of the lipid materials and drugs in chloroform (Table 1), the organic solvent was removed using Rotavapor (Heidolf VI 2000) until a thin lipid film on the wall of the flask was obtained. Overnight drying in a desiccator under vacuum was performed to eliminate any residual traces of solvent. Subsequently, the lipid film was hydrated with 1 mL of PBS pH 7.4 for 1 h in a thermomixer under mild stirring (37 ºC, 300 rpm). After this process, three freeze & thaw cycles were performed in liquid nitrogen (-196 ºC) and in

Thermomixer (37 ºC) respectively, to obtain a homogenous liposomal suspension. The drug-loaded liposomes were sonicated (Omni Ruptor 250, Omni International Inc, Ultrasonic homogenizer) for 90 min. For the separation of the free drug and evaluation of drug entrapment efficiency, all liposomal formulations were chromatographed over a PD-10 desalting column (GE Healthcare, USA).

Evaluation of encapsulation efficiency (EE)

An aliquot of liposomes was disrupted by mixing the liposomal suspension with absolute ethanol (1:5 v/v), vortexing at high speed for 5 min, and then centrifuging at 4000 ×g for 1 min. The supernatant was analyzed by dynamic light scattering (DLS) (Malvern Zeta Sizer) to ascertain membrane disruption. The amount of drug encapsulated in the liposomes was determined with reference to a calibration curve of lumefantrine standard solutions and the encapsulation efficiency (EE) was obtained as in Eq 1.

EE (%) = (Ac/Tc)100 ……. (1)

where Ac and Tc are the actual and theoretical drug contents, respectively.

Particle size and polydispersity index of liposomes

Dimensional analysis was performed by DLS to determine the particle size distribution profile and polydispersity index of all the prepared formulations.

Quantitative determination of phospholipids by Stewart assay

Stewart assay was used to determine the molarity of the phospholipids in the liposomal formulations. Standard solutions for the calibration plot were obtained by different dilutions of a stock solution of 0.1 mg/mL of Lipoid S75 in chloroform at a final volume of 2 mL each and further addition of 2 mL of ammonium ferrothiocyanate. Similarly, a small aliquot of liposomes was diluted into 2 mL of chloroform and 2 mL of reagent was added. The solutions were stirred on a vortex (VelpScientifica, Advanced Vortex mixer) for 20 s and then centrifuged at 1000 rpm for 10 min. The lower clear organic phase was removed using a Pasteur pipette and the absorbance was determined at λ 485 nm (Evolution 201 UV-VIS Spectrophotometer, ThermoScientific, USA).

Stability studies

The lumefantrine-loaded liposomes were subjected to Triton X-100 titration and monitored by DLS measurements. Sequential additions of 10 µL of a surfactant solution of Triton X-100 (50 mM) were added into single-use polystyrene cuvettes with a path length of 10 mm, containing 170 µL of 3 mM liposomal suspension. All experiments were performed at 37 ºC. Liposomes' average size after each addition of Triton X-100 was measured following an equilibration time of 900 sec. Each sample was analyzed by recording three measurements of 10 runs each. The data were reported as mean ± standard deviation (SD, n = 3).

Short-term stability studies

Samples of 150 µL (3mM) of lumefantrine-loaded CL, SL, or SSL containing 0.05 % w/v of sodium azide (NaN_3), were stored at 4 ºC, room temperature and 37 ºC over a period of 72 h. DLS was used to ascertain the stability after 24 and 72 h.

***In vivo* antimalarial activity in mice**

The percent parasitaemia reduction of lumefantrine, encapsulated in the different liposomal formulations, was assessed by in-vivo. The mice were divided into five groups of six mice each. At the beginning of the test, percentage parasitemia and red blood cell count of the experimental mice were established. Whole blood was collected from the donor mouse by puncturing the bleeding heart and diluting with normal saline to a concentration of 10^8 parasitized erythrocytes/ml. All 30 mice were inoculated with 0.2 ml of the 10^8 parasitized erythrocytes intraperitoneally (i.p) on day 1 and left untreated until the fourth day (D4) post–inoculation (17).

Table 1: Molar ratios for CL, SL, and SSL

Treatment group	Lipoid S75	Cholesterol	DSPE	Lumefantrine	$mPEG_{5000}$-DSPE	$mPEG_{5000}$-βGlu-$(DSPE)_2$
CL	6	1	1	1.5	-	-
SL	6	1	1	1.5	0.5	-
SSL	6	1	1	1.5	-	0.5

After inoculation, all treatments were given orally per day for 3 days (D4–D6). Group A mice (untreated group) were given 0.2 mL/kg of normal saline, while Group B (CL), C (SL) or, D (SSL) mice received 7.5 mg/kg body weight of lumefantrine equivalent through the tail vein. Group E (positive control) received 5 mg/kg of chloroquine injection through the tail vein. On day 7 and 13, blood was collected from the tail-vein and smeared on a miscroscope slide. The thin blood films were mixed with 10 % Giemsa solution and examined microscopically to assess parasitaemia level.

$P\ (\%) = (P_o\text{-}P_f/P_o) \times 100$……. (2)
where P = parasitaemia reduction, Po = parasitaemia prior to treatment, and Pf = parasitaemia after treatment.

Statistical analysis

The percent parasitaemia reduction was expressed as the mean value ± SD. One-way analysis of variance (ANOVA) of Sigma Plot 11.0 was used to determine the statistical differences between the groups. $P < 0.05$ was considered statistically significant.

RESULTS

Characterization of $mPEG_{5000}$-$(DSPE)_2$

The synthesized PEG Dendron;$mPEG_{5000}$-$(DSPE)_2$, was characterized by ^1H NMR spectroscopy (400 MHz) in $CDCl_3$ and confirming that two DSPE molecules were coupled per polymer chain (Figure 1). ^1H-NMR spectroscopy (400 MHz) of $mPEG_{5000}$-$(DSPE)_2$ in $CDCl_3$: δ 3.64 (s, 459.89H, -O-CH_2- PEG), 3.38 (s, 3H, -O-CH_3 PEG), 1.25 (m, 12.74H, -CH_2- DSPE), 0.87 (t, 13.66H, -CH_3 DSPE).

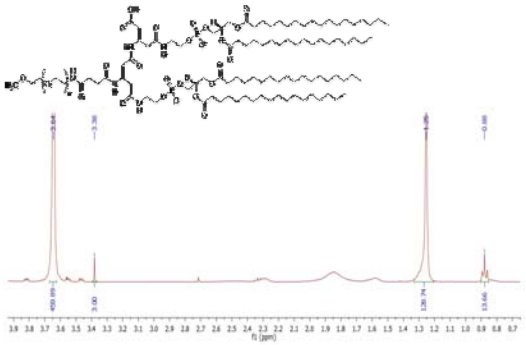

Figure 1: Chemical structure and ^1H NMR spectroscopy of $mPEG_{5kDa}$-$(DSPE)_2$ in $CDCl_3$

Particle size and polydispersity index

The mean particle sizes of drug-loaded liposomes were between 235 and 248 nm. The sizes and PdI decreased after sonication except the classical liposomes (Table 2).

Encapsulation efficiency

The encapsulation efficiencies of the CL, SL, and SSL were 44.4, 42.5, and 66.0 %, respectively. The SSL had the highest incorporation of the hydrophobic lumefantrine.

In vitro stability

The liposomal vesicles increased in particle size as the volume of 50 mM Triton X-100 increased until destabilization. The first to be destabilized was the SSL < CL < SL (Table 3).

Table 2: Particle size and PdI of the CL, SL, and SSL

Formulation	Before sonication and gel filtration		After sonication and gel filtration	
	Particle size (nm)	*PdI*	*Particle size (nm)*	*PdI*
Lumefantrine CL	201.2 ± 57.85	0.64 ± 0.04	248.0 ± 44.89	0.78 ± 0.02
Lumefantrine SL	315.7 ± 76.4	0.468 ± 0.05	235.83 ± 45.13	0.39 ± 0.06
Lumefantrine SSL	270.6 ± 97.48	0.56 ± 0.16	238.2 ± 23.0	0.24 ± 0.04

Table 3: Triton X-100 titration data for liposomes

Volume of Triton X-100 (µL)	CL		SL		SSL	
	Particle size (nm)	*PdI*	*Particle size (nm)*	*PdI*	*Particle size (nm)*	*PdI*
10	679.2±108	0.19±0.06	470.8±53.8	0.15±0.07	420.8±60.1	0.24±0.02
20	824.2±105.6	0.10±0.07	628.2±90.1	0.25±0.01	594.4±99.9	0.38±0.12
30	1026.8±294.2	0.24±0.10	788.2±322.7	0.14±0.19	616.1±557.7	0.47±0.06
40	1130±109.6	0.219±0.03	1175.1±312.9	0.33±0.06	10.55±0.36	0.53±0.36
50	830.76±712.0	0.25±0.05	1158.6±137	0.13±0.11	-	-
60	10.19±1.02	0.24±0.16	1178.3±151.0	0.18±0.09	-	-
70	-	-	2016.3±143.1	16183±53.2	-	-
80	-	-	1285.6±325.5	0.59±0.46	-	-

Table 4: Stability of liposomes after incubation with sodium azide at different temperatures

Liposome type	$T_{24\ h}$		
	4 ºC	*25 ºC*	*37 ºC*
CL	752.2 ± 177.5	632.23 ± 49.1	854.1 ± 16.3
	0.24 ± 0.05	0.18 ± 0.06	0.29 ± 0.04
SL	363.7 ± 29.9	346.7 ± 51.6	421.9 ± 168.8
	0.24 ± 0.01	0.24 ± 0.03	0.27 ± 0.08
SSL	309.2 ± 31.7	300.1 ± 51.5	238.2 ± 23.09
	0.22 ± 0.008	0.23 ± 0.01	0.24 ± 0.04
	$T_{72\ h}$		
	4 ºC	*25 ºC*	*37 ºC*
CL	442.4 ± 307.4	570.6 ± 406.3	573.4 ± 465.2
	0.29 ± 0.05	0.41 ± 0.02	0.264 ± 0.03
SL	337 ± 64.5	312.7 ± 46.03	313.7 ± 168.7
	0.25 ± 0.03	0.18 ± 0.04	0.25 ± 0.04
SSL	372. 4 ± 98.9	287.6 ± 66.2	307.2 ± 89.4
	0.54 ± 0.06	0.19 ± 0.06	0.31 ± 0.02

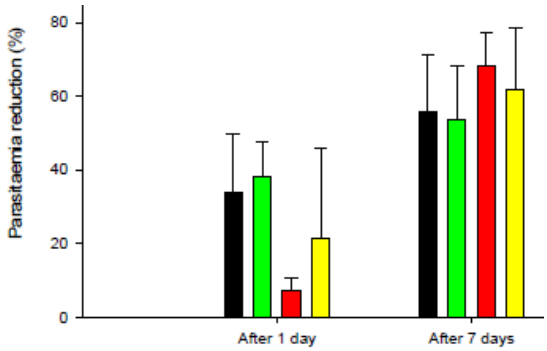

Figure 2: Reduction (%) of parasitaemia in mice after challenge with *Plasmodium berghei* treated with lumefantrine- loaded liposomal formulations. Key: Black =CL; Green = SL; Red-= SSL; Yellow = Chloroquine (positive control)

Short-term stability

Sodium azide was added to avoid bacterial growth during incubation. The stability of drug-loaded CL, SL, or SSL stored at 4 °C, room temperature (RT), and 37 °C was assessed after 24 and 72 h (Fig. 3). Interestingly, CL formulation showed evidence of aggregation already after 24 h of incubation even at the lowest temperature of 4 °C while SL and SSL formulations of lumefantrine showed high stability with minimal variation of the initial sizes at 4 and 37 °C and after 72 h (Table 4).

Pharmacodynamic data

Liposomal formulations of lumefantrine were tested in a mouse model for antimalarial activity. Chloroquine was used as the positive control because *Plasmodium berghei* is known to be sensitive to chloroquine. After the 3 days treatment, the results showed that the parasitaemia reduction was less than 40 % in all the groups. After 7 days, post treatment there was a significant percent reduction in parasitaemia at $p < 0.05$ by SSL (68.3 ± 8.9) followed by chloroquine (61.9 ± 16.7), CL (55.8 ± 15.2), and SL (53.4 ± 14.9), shown in Figure 2.

DISCUSSION

Classical liposomes are spherical self-closed vesicles consisting mainly of phospholipids and cholesterol. In this study, we used Lipoid S75. A major limitation of the classical liposomes in drug delivery is opsonization and this led to the pegylation of liposomes called stealth liposomes. Pegylation shields liposomes from the reticuloendothelial system and prolongs circulation. We used methoxy polyethylene glycol distearoyl phosphoethanolamine ($mPEG_{5000}$-$DSPE)_2$ for the pegylation but polyethylene glycol detaches from the surface of liposomes when plasma proteins interact with the polymer leaving the liposomes vulnerable to opsonization. Hence, the synthesis of super-stealth liposomes in which β-glutamine is incorporated into the structure, thereby strengthening the interaction between the phospholipid bilayer and the methoxy polyethylene glycol [18,19].

For parenteral delivery of lumefantrine encapsulated in liposomes, the particle size and polydispersity index was relatively high. In liposomes, particle size of 100 nm and PdI of 0.3 or below is considered acceptable and indicates a homogenous population of phospholipid vesicles [24]. The low value of PdI is also an indication of a stable formulation [20-24]. The larger particle sizes of the loaded liposomes might be attributed to the presence of lumefantrine that, being hydrophobic, accumulate in the liposome phospholipid bilayer. It is also possible that $mPEG_{5000}$-βGlu-$(DSPE)_2$ helped to incorporate higher amounts of lumefantrine within the bilayer rather than being adsorbed on

the surface, thus producing a more homogenous population of vesicles for the SSL. SSL showed higher encapsulation efficiency and further investigations are needed to evaluate if $mPEG_{5000}$-βGlu-$(DSPE)_2$ has a role in improving the loading of other hydrophobic drugs in the liposome phospholipid bilayer. This higher encapsulation of lumefantrine in SSL possibly enhanced the percent reduction of parasitaemia in the rats. In-vivo stability is a pre-requisite in the design of liposomes. The disrupting action of Triton X-100 is a function of lipid composition of the liposome, Triton concentration, temperature and duration of detergent incubation [22].

Increasing the volume of the detergent increased the particle sizes of CL, SL and SSL steadily owing to the incorporation of the detergent in the phospholipid bilayer until the vesicles were disrupted at their solubilization boundary [23]. It is possible that the hydrophobic lumefantrine, incorporated at higher amounts in SSL, interfered with the stability of the liposomes. It is evident that storage at higher temperatures had an effect on the physical size of liposomes as higher temperatures caused aggregation of the liposomes. For the pharmacodynamic study, although the percent reduction of parasitaemia for SSL at 7 days was not statistically significant at $p < 0.05$ with respect to chloroquine (positive control) it is worth noting the reduction of intra-sample variability in the case of SSL formulation. The higher response of SSL to parasitaemia reduction could be due to its known prolonged pharmacokinetic profile, thus achieving a higher drug exposure. Furthermore, SSL already displayed superior activity with respect to CL and SL when delivering other drugs and it was also related to higher cell internalization [23,25,26].

CONCLUSION

This preliminary study shows that SSL encapsulated a higher amount of lumefantrine and presented increased stability at all the test temperatures with respect to CL or SL. Therefore, this liposomal formulation is a promising therapeutic formulation for malaria, but further development studies ae required to optimize both the phospholipid composition and drug loading, possibly in combination with another artemether to achieve a stronger activity against malaria.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ebele Onuigbo, Gianfranco Pasut, Anthony Attama and Elena Canato designed the work, Gianfranco Pasut and Elena Canato supervised it while Ebele Onuigbo and Gianfranco Pasut collected and analyzed the data and prepared the manuscript, which was then approved by all authors.

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REFERENCES

1. *Attama AA, Kenechukwu FC, Onuigbo EB, Nnamani PO, Obitte N, Finke JH, Pretor S, Muller-Goymann CC. Solid lipid nanoparticles encapsulating a fluorescent marker (Coumarin 6) and antimalarials- artemether and lumefantrine: evaluation of cellular uptake and antimalarial activity. Eur J Nanomed 2016; 8: 51-59.*
2. *Varela M, Mbengue B, Basse A, Loucoubar C, Vigan-Womas I, Dieye A, Toure A, Perraut R. Optimization of a magnetic bead-based assay (MAGPIX®-Luminex) for immune surveillance of exposure to malaria using multiple Plasmodium antigens and sera from different endemic settings. Malaria J 2018; 17: 324.*
3. *Costa Cesar I, Nogueira FH, Pianetti GA. Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection. J Pharm Biomed Anal 2008; 48: 951-954.*
4. *Nnamani PO, Hansen S, Windbergs M, Lehr CM. Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application. Int J Pharm 2014; 477: 208-217.*

5. Agbo C, Umeyor C. Kenechukwu F, Ogbonna J, Chime S, Lovelyn C, Agubata O, Ofokansi K, Attama AA. Formulation design, in vitro characterization and antimalarial investigations of artemether and lumefantrine-entrapped solid lipid microparticles. Drug Dev Ind Pharm 2016; 42: 1708-1721.
6. Jayakrishnan L, Sudhikumar AV, Aneresh EM. Role of gut inhabitants on the vectorial capacity of mosquitoes. J Vector Borne Dis 2018; 55: 69-78.
7. Bandyopadhyay S, Luma L, Kroeger A. Classifying dengue haemorrhagic fever. Trop Med Int Health 2006; 8: 1238-1255.
8. Singhvi G, Pahl V, Gupta G, Hansbro PM, Dua K. Microbiome as therapeutics in vesicular delivery. Biomed Pharmacother 2018; 104: 738-741.
9. Hoglund R, Ruengweerayut R, Bangchang K. Population pharmacokinetics of mefloquine given as a 3-day artesunate–mefloquine in patients with acute uncomplicated Plasmodium falciparum malaria in a multidrug-resistant area along the Thai–Myanmar border. Malaria J 2018; 17: 322.
10. Desormeaux A, Bergeron MG. Liposome as a drug delivery system: a strategic approach for the treatment of HIV infection. J Drug Targ 1998; 6: 1-15.
11. Jin SX, Wang DZ, Wang YZ, Hu Deng YG. Pharmacokinetic and tissue distribution of Zidovudine in rats following intravenous administration of Zidovudine myristate-loaded liposomes. Pharmazie 2005; 60: 840-843.
12. Verma A, Jain A, Tiwari A, Saraf S, Panda PK, Agrawal GP, Jain SK. "Folate Conjugated Double Liposomes Bearing Prednisolone and Methotrexate for Targeting Rheumatoid Arthritis", Pharmaceut Res, 2019; 36(8):123. doi: 10.1007/s11095-019-2653-0.
13. Mkabi-Panze B, Gourde P, Desormeaux A, Bergeron MG. Intracellular and serum stability of liposomal 2,3-deoxycytidine: effect of lipid composition. Cell Mol Biol 1998; 44: 277-284.
14. Sharma A, Sharma US. Liposome in drug delivery: progress and limitations. Int J Pharm 1997; 154: 123-140.
15. Pattni BS, Chupin VV, Torchillin VP. New Developments in Liposomal Drug Delivery. Chem Rev 2015; 115: 10938-10966.
16. Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle K, Moyes CL, Henry A, Eckhoff PA, Wenger EA, Briet O, Penny MA, Smith TA, Bennett A, Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray CLJ, Smith DL, Hay SI, Cibulskis RE and Gething PW. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2005. Nat 2015; 526: 207.
17. Ong S, Ming L, Lee K, Yuen K. Influence of the encapsulation efficiency and size of liposome on the oral bioavailability of Griseofulvin-loaded liposomes. Pharmaceut 2016; 8(3): 25. Doi10.3390/pharmaceutics8030025.
18. Peters W, Ze-Lin L, Robinson BL, Warhurst DC. The chemotherapy of rodent malaria XL. Am Trop Med Parasitol 1986; 80: 483-489.
19. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceut 2018; 10(2): 57.
20. Pasut G, Paolino D, Celia C, Mero A, Joseph A, Wolfram J, Cosco D, Schiavon O, Shen H, Fresta M. Polyethylene glycol(PEG)-dendron phospholipids as innovative constructs for the preparation of super stealth liposmes for anticancer therapy. J Contr Rel 2015; 199: 106-113.
21. Manosroi A, Khanrin P, Lohcharoenkal W, Werner RG, Manosroi W, Götz F, Manosroi W, Manosroi J. Transdermal absorption enhancement through rat skin of gallidermin-loaded in niosomes. Int J Pharm 2010; 392: 304–310.
22. Sila M, Au S, Weiner N. Effects of Triton X-100 concentration and incubation temperature on carboxyfluorescein release from multilamellar liposomes. Biochimica et Biophysica Acta (BBA) – Biomembranes 1986; 859(2): 165-170.
23. Muzzalupo R, Tavano L, Trombino S, Cassano R, Piccia N, La Mesab C. Niosomes from α,w-trioxyethylene-bis(sodium2-dodecyloxy-propylenesulfonate): preparation and characterization. Coll Surf B: Biointer 2008; 64: 200–207.
24. Danaei M, Dehghankhold S, Ataei D, Hasanzadeh R, Javanmard A, Dokhani S, Khorasani Mozafari M. Impact of particle size and polydispersity index on the clinical applications of lipid nanocarrier systems. Pharmazie 2018; 10: E57.
25. Catanzaro D, Nicolosi S, Cocetta V, Salvalaio M, Pagetta A, Ragazzi A, Montopoli A, Pasut G. Cisplatin liposome and 6-amino nicotinamide combination to overcome drug resistance in ovarian cancer cells. Oncotarget 2018; 9: 16847-16860.
26. Gabbia D, Canato E, Carraro V, Tomasini L, Guido M, Pasut G, De Martin S. Novel super stealth immunoliposomes for cancer targeted delivery of doxorubicin: an innovative strategy to reduce liver toxicity. Dig Liver Dis 2019; 51:21.