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ARTICLE

Synthesis, Characterization and Evaluation of Lecithin Based Nanocarriers for Enhanced Pharmacological and Oral Pharmacokinetic Profile of Amphotericin B

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Ibrahim Javed[a,f], Syed Zajif Hussain[a], Irfan Ullah[b], Imran Khan[c], Muhammad Ateeq[d], Gul Shahnaz[e], Habib ur Rehman[a], Muhammad Tahir Razi[f], Muhammad Raza Shah[d] and Irshad Hussain[a*]

We report the synthesis, characterization and evaluation of lecithin-drug hybrid nanocarriers (NCs) with enhanced oral bioavailability and anti-parasitic potential of poorly water soluble drugs. Amphotericin B (AmB), a poor water soluble drug with poor membrane penetrating ability, was selected as a model drug to demonstrate the potential of the lecithin-drug hybrid NCs. Lec-AmB NCs were prepared by the self-assembly of lecithin into nanoparticles (NPs) at a critical micellar concentration of 4mg/ml and into liposomes at a critical liposomal concentration of 53mg/ml in aqueous systems. Lec-AmB NPs (200-300 nm) were further coated with polyethylene glycol (MW 600) and Tween 20 while liposomes (70-90 nm) were used as such for this study. Lec-AmB NCs were evaluated for their ability to boost in-vivo oral pharmacokinetic parameters in rabbits and in-vitro anti-leishmanial activity against promastigotes of *Leishmania tropica*. A reciprocal relationship was observed between the size and drug encapsulation efficiency of NPs but no such relationship was witnessed in case of liposomes. More importantly, the oral bioavailability and anti-leishmanial activity of Lec-AmB NPs was enhanced up to 21 and 6.3 folds, and 21 and 2 folds respectively in case of liposomes. The improvement in the bioavailability and anti-leishmanial activity is very significant compared to the deoxycholate complex of AmB (water soluble, injectable market product: Anfotericina FADA®), and this study thus represents the promising potential of easy-to-prepare NCs with improved therapeutic efficiency using phosphocholine based biocompatible surfactants.

1. Introduction

Amphotericin B (AmB), a polyene antifungal, is one of the most promising therapeutic agents against systemic fungal infections. It has also been evaluated against leishmaniasis and demonstrated a good clinical potential. The results are especially impressive when AmB is given to immunocompromised cancer patients, suffering from opportunistic systemic or even meningeal fungal infections.[1, 2] Various anti-leishmanial drugs have been evaluated including prominent antimony compounds which ended up with irreversible renal failure, antimony associated toxicities and drug resistance in leishmanial strains.[3, 4] Currently, AmB represents the most effective and safe candidate for the treatment of visceral leishmania.[5] However, due to its physicochemical properties, AmB is placed in Class 4 of Biopharmaceutical Classification System (BCS) because of its poor water solubility and membrane permeability.[6] It results in poor oral bioavailability and instability in gastric acidic environment thus limiting the effective clinical applications of AmB. The parenteral dosage forms of AmB, currently available in the market, are associated with nephrotoxicity and erratic systemic bioavailability.[1] A drug needs to be soluble in aqueous gastric media, prior to its absorption in systemic circulation, across gastric membrane. The formulation of AmB offering optimum control over pharmacokinetic parameters with minimum renal toxicity, without compromising its excellent anti-fungal and anti-leishmanial potential is thus highly desirable. Nanoscale pharmaceutical formulations offer a drug development tool to overcome the problems associated with such physicochemical properties of drugs. Previously synthetic polymer based nanocarriers have been reported to reduce nephrotoxicity and hemolytic activity while improving oral bioavailability and anti-parasitic activity of AmB.[7-9] These formulations, however, usually result in reduced anti-parasitic activity or low drug loading. The development of hydrophilic, biodegradable and biocompatible nanoparticulate formulations are, therefore, highly desired to improve the

[a]*Department of Chemistry, SBA School of Science & Engineering (SSE), Lahore University of Management Sciences (LUMS), DHA, Lahore Cantt 54792, Pakistan.*
[b]*Department of Pharmacy, University of Peshawar, Nathia Gali-Abbottabad Road, Bara Gali, Bagnotar 25120, Pakistan.*
[c]*Food Science and Biotechnology, School of Bioconvergence Science and Technology, Kangwon National University, Chuncheon, Gangwon 200–701, Republic of Korea.*
[d]*H.E.J Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan.*
[e]*Department of Pharmacy, Quaid-i-Azam University, Islamabad 45320, Pakistan.*
fDepartment of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University, Bosan Road, Multan 60000, Pakistan.
[*]*Corresponding author: Irshad Hussain, Department of Chemistry, SBA School of Science & Engineering (SSE), Lahore University of Management Sciences (LUMS), DHA, Lahore Cantt 54792, Pakistan.*
Tel.: +92 (42) 35608133
E-mail address: ihussain@lums.edu.pk (Irshad Hussain)

bioavailability and anti-parasitic activity of drugs. The use of food-grade biocompatible materials to address the issues associated with the bioavailability, toxicity and pharmacokinetic parameters of drugs like AmB, offers obvious advantages over the synthetic materials like polylactide co-glycolic acid or polycaprolactones.

In this study, soya lecithin (Lec) based biodegradable and water soluble nanocarriers of AmB (Lec-AmB NCs) in the form of nanoparticles (NPs) and liposomes were prepared, characterized and evaluated for their oral bioavailability in rabbits and anti-leishmanial potential against *Leishmania tropica*. Surface of Lec-AmB NPs was modified with polyethylene glycol (MW 600) and Tween 20 to further improve their aqueous solubility, stability and biological activity. Both of these biocompatible NCs demonstrated their remarkable potential to address the challenges associated with the stable oral bioavailability of AmB and to significantly enhance its anti-leishmanial activity. [3]

2. Experimental Procedures

2.1. Materials

Soya lecithin (L-α-phosphatidylcholine MW = 750) and Amphotericin B (European pharmacopoeia reference standard) were purchased from Sigma Aldrich. Tween 20 (polyoxyethylene sorbitanmonolaurate polysorbate) and polyethylene glycol (MW = 600) were purchased from Merck. Anfotericina FADA® (Amphotericin B-deoxycholate) was purchased from FADA Pharma, Argentina, through Medinet Pharmaceuticals, Pakistan. All chemicals were used as such without further purification. All organic solvents used, were of Merck analytical grade.

2.2. Synthesis of Lecithin Nanoparticles

Self-assembled NPs of lecithin were prepared via one-pot nano-emulsification technique. [10] Briefly, lecithin was initially dissolved in chloroform and misciblized with solution of AmB in methanol and chloroform (1:4) at different concentrations. Solvents were then evaporated by gentle heating under vacuum in dark to avoid photo degradation of AmB. The homogenous mixture of lecithin and AmB was then re-dispersed in deionized water to obtain 4 mg and 5 mg lecithin/ml of water. After 10 min of stirring, when lecithin-AmB dispersion turned opaque, PEG 600 and Tween 20 (1 %, w/v) were added separately and drop-wise to the system. A less opaque, but not clear, suspension was obtained at the end indicating the formation of lecithin micelles entrapping AmB.

2.3. Synthesis of Lecithin Liposomes

Lecithin liposomes were prepared by employing a reported method with slight modification. [11] Starting with the same procedure as for NPs, the dried mixture of lecithin and AmB was re-dispersed in water to obtain final concentration of 53 mg/ml of lecithin (critical liposomal concentration) and 1.6 or 3.2 mg/ml of AmB in water. The resulting formulation was magnetically stirred for 3 - 4 h under N2 atmosphere and then sonicated for 15 min with 0.5 second on/off cycle. It resulted in the formation of less opaque liposomal formulations, which were stored in dark at room temperature to avoid photodegradation of AmB. Figure 1 shows the scheme of the synthesis of both of these hybrid lecithin-drug NCs.

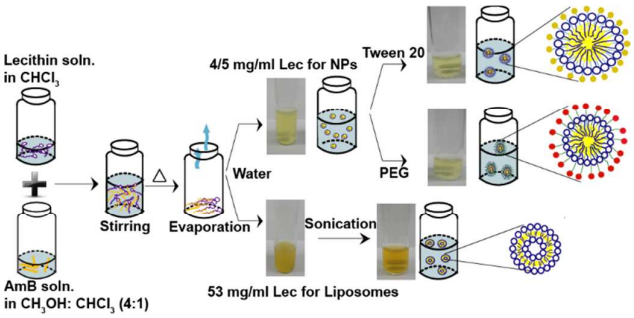

Fig. 1 Synthetic scheme for the formation of Lec-AmB NPs and liposomes.

2.4. Encapsulation Efficiency

Different concentrations of AmB were entrapped in lecithin NPs (0.05-1.2 mg/ml) and liposomes (1.6 or 3.2 mg/ml). Encapsulation efficiency was determined by removing the loosely bound/free drug on lecithin NPs and liposomes by centrifuging the final NPs/liposomal suspension at 8000 rpm / 4 °C for 10 min. Un-entrapped water insoluble drug settled down as a pellet and the concentration of drug entrapped in lecithin NPs/liposomes, suspended in supernatant, was quantified by measuring the absorbance of AmB at 406 nm. [12] For this purpose, lecithin NPs/liposomes were diluted in a mixture of chloroform: methanol (1:4) and rigorously sonicated for 10 min to rapture the NPs/liposomes and release all the drug contents in solvent system. Encapsulation efficiency was then calculated using the mathematical expression below:

Encapsulation efficiency (%) = (drug in supernatant after rupturing of NPs / liposomes) / (total drug added to system) × 100

2.5. Morphology and Zeta-Potential of Nanocarriers

Hydrodynamic radius and zeta potential of Lec-AmB NCs, dispersed in water, was measured with Zetasizer (Malvern, Nano ZSP). STEM images were captured by Scanning Electron Microscope (FEI Nova NanoSEM 450) equipped with transmission electron detector, operated at 15 KV. Samples for transmission electron microscopic analysis were prepared by slow evaporation of a drop of Lec-AmB NCs on a carbon coated copper grid, followed by blotting with a drop of 1 % ammonium molybdate solution.

2.6. XRD, DSC and FTIR Analysis of Nanocarriers

The amorphous nature of drug inside nano-formulations was evaluated by powder X-ray Diffractometer (Bruker, D2 Phaser) and differential scanning calorimeter (TA instruments, SDT Q600). The XRD patterns were collected by X-ray Diffractometer operated at angle range of 10-70° with step size of 0.02° and scanning rate of 4°/min. DSC results were obtained at 50-300 °C with a heating rate of 20 °C/min under N_2 purge of 30 ml/min. The stability of drug and its functional groups inside NPs was confirmed with FTIR (Bruker alpha-P). All these characterizations were performed for pure drug, freeze dried Lec-AmB NPs, AmB liposomes and physical mixture of drug with empty NCs.

2.7. Oral Pharmacokinetics

Oral bioavailability studies were performed on local species of rabbits (Oryctolagus cuniculus). Ethical guidelines, set by EU directive 2010/63, were followed in designing and conducting the study on animals. [13] Non-compartmental approach was used to

access pharmacokinetic parameters. [14] For this purpose, animals were housed under standard conditions i.e. at 25 °C and 12 h day-night cycle with free access to fodder and water. AmB dispersion and solution of AmB-deoxycholate in distilled water were used as positive control. Animals were divided into 5 groups (n = 4). Single oral dose of AmB (4mg/kg) of control (crystalline AmB and Anfotericina FADA®) and all other nano-formulations having equivalent amount of AmB were given orally. Blood samples were collected in heparinized tubes via marginal ear vein. Plasma was separated and stored at -80 °C until further analysis.

2.7.1. Plasma Drug Extraction and HPLC Conditions

Drug was extracted from plasma by protein precipitation and quantified by HPLC (Shimadzu, LC20A). For this purpose, plasma (200 µl) was vortex with methanol (100 µl) for 3 min and centrifuged at 12,000 rpm. Clear supernatant was collected, vacuum concentrated and reconstituted in 150 µl mobile phase, 100 µl of which was injected into HPLC column (Lichrocart C8, 120×4.6mm, 5µm). Mobile phase, consisting of acetonitrile and 0.05 N sodium phosphate buffer (34:66), was delivered iso-cratically at a rate of 1 ml/min and the absorbance of eluent was measured at 406 nm. [15]

2.8. Anti-leishmanial Activity

In-vitro anti-leishmanial activity was performed on promastigotes of Leishmania tropica KHW23 strain [16], which was incubated at 24 °C till a culture with concentration of 1 x 10^6 promastigotes/ml was achieved. In order to find MIC, activity was performed against different AmB equivalent concentrations of each formulation. Amphotericin B deoxycholate solution in water was used as positive control. The plate was incubated at 24 °C for 72 h and mortality was recorded by counting live promastigotes in neubar counting chamber.

Statistical analysis

The data is represented as mean with ± standard deviation (SD). Differences in results of test samples from control, for oral bioavailability parameters and anti-leishmanial activity, were accessed for significance by one way analysis of variance (ANOVA) followed by Tukey test. A P value of <0.05 was considered statistically significant.

3. Results and Discussion

Amphiphilic lecithin molecules has an intrinsic property of self-assembling into micelles. [17] Critical micelle concentration of lecithin is about 4 mg/ml but when its concentration is increased to about 53 mg/ml in water, it forms liposomes. [11] This enables lecithin as an attractive building block to prepare biocompatible nano-carriers for drug delivery applications. In this study, AmB, a hydrophobic drug, was self-assembled with lecithin and this physicochemical complex was reconstituted in water to form NPs or liposomes. The drug is entrapped inside the core and bilayer assembly of NPs and liposomes respectively. NPs were further decorated with PEG 600 and Tween 20 in order to enhance the stability of lecithin based NPs in water. According to FDA, lecithin is safe, integral part of food for appropriate control over lipid profile and has not shown any adverse effects up to a dose of 20 g/day in humans.[18]

While preparing the NCs lecithin acted as an encapsulating agent for drug. Average particle size of about 250 nm in case of Lec-AmB Tw NPs and 300 nm in case of Lec-AmB PEG NPs was obtained, while liposomes were in the size range of 70-90 nm (Figure 2).

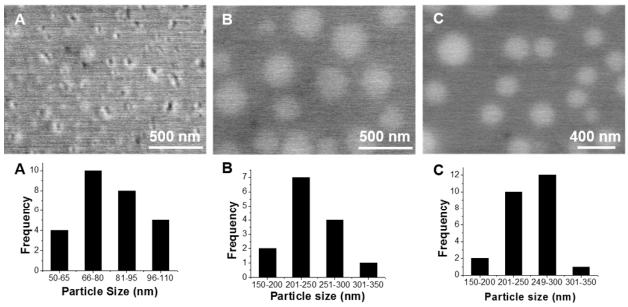

Fig. 2 Scanning electron micrographs of AmB lecithin NPs, liposomes and their respective size distribution histograms. (A) AmB-Lec Liposomes, (B)AmB-Lec Tw NPs and (C) AmB-Lec PEG NPs.

Particle size distribution was found to be dependent on the amount of encapsulated drug. Increasing the drug concentration resulted in increased encapsulation efficiency and decrease in the particle size, but up to a certain limit beyond which the particles size started increasing by increasing the amount of encapsulated drug. It may be explained on the basis of saturation point of lecithin particles for drug uptake. Before that saturation point, increased amount of drug was able to get entrapped inside particles and lecithin was available to make more particles to entrap the drug instead of depositing on the same particles to thicken the lecithin layer in them. After saturation point of lecithin NPs for AmB, increasing the drug concentration didn't significantly increase the entrapped amount of drug i.e. decreased encapsulation efficiency, but increased the particle size instead by depositing in surface pockets. Maximum encapsulation efficiency with smaller sized particles was achieved around the saturation point (Figure 3).

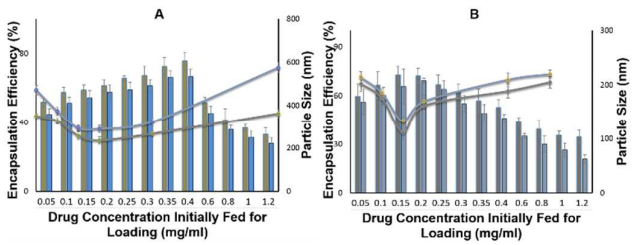

Fig. 3 Effect of initially fed drug concentrations on particle size (line graph) and encapsulation efficiency (bars graph) of AmB-Lecithin NPs (n=3, mean±SD) with 4mg/ml (blue) and 5mg/ml (red) of Lecithin. (A) AmB-Lec Tw NPs and (B) AmB-Lec PEG NPs.

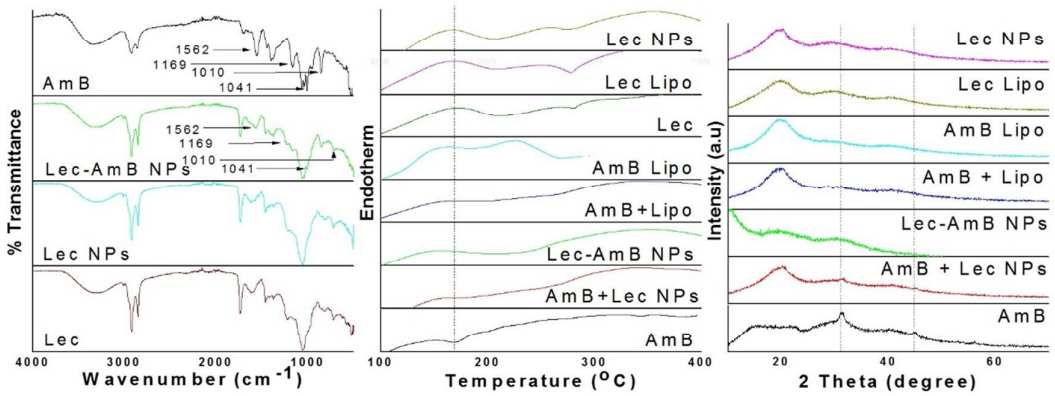

Fig. 4 XRD, DSC and FTIR results of AmB, Lecithin, empty Lec-NPs and Liposomes, Lec-AmB NPs and Liposomes and physical mixture of empty Lec-NPs and Liposomes with AmB.

Increasing the lecithin concentration from 4 to 5 mg/ml produced more particles for drug entrapment and the same trend was observed but with enhanced drug uptake efficiency. It indicates that drug entrapment can be tuned to the desired dose by lecithin concentration. The trend was same in both PEG and Tween coated NPs, but due to long alkyl chains of PEG (MW-600) on particle surface, greater hydrodynamic radius was observed as compared to Tween coated particles. In case of liposome, the particle size and amount of drug entrapped was not affected by increasing the amount drug to twice. This may be due to the fact that liposomes are able to accommodate more amount of drug in its bilayer structure, without any significant change in their size (Table 1).

Table 1. Effect of initially fed drug concentration on particle size and encapsulation efficiency of liposomes (Mean ± SD)

Initially Fed AmB (mg/ml)	**1.6**	**3.2**
Entrapped AmB (mg/ml)	0.23 ± 0.09	0.24 ± 0.06
Size (nm)	80 ± 3	95 ± 2

Physical characteristics of nanocariers were further studied by their characterization with FTIR, DSC and XRD (Figure 4). Diffraction peaks of Lecithin-AmB NCs observed at 32 and 45 degree were diminished in the physical mixture of AmB and empty nano-carriers (particles and liposomes) while completely disappeared in Lec-AmB NCs. Likewise, characteristic DSC thermogram of crystalline AmB was observed with a peak at 170 °C which was absent in AmB nano-formulations and instead of peak, a slight straight pattern is observed in physical mixtures of crystalline AmB and empty NCs. DSC and XRD analysis revealed amorphous nature of drug inside NPs, which indicates better solubility in aqueous media. Characteristics FTIR spectrum peaks of AmB are observable at 1010 (trans polyene chromophore), 1041 (NH_2), 1169 (C-O-C of β-glycosidic linkage) and 1562 cm^{-1} (NH_3^+)[19] which are also present in Lec-AmB NPs, indicating the stability of drug functionalities inside NPs. The prepared Lec-AmB NPs and liposomes were physically stable up to 6 months in dark at 4 °C with no significant degradation of drug contents.

Physical mixtures were prepared according to drug loading ratio of NCs. These results indicate that all of drug was entrapped inside core and bilayer of particles and liposomes. FTIR analysis showed the presence of AmB in both the NCs exhibiting no significant difference in the IR absorption peaks of lecithin functional groups, whether in free form or encapsulated inside NCs.

Lec-AmB NCs were, in fact, designed primarily to improve the oral bioavailability of drug, which was evaluated in healthy rabbits of either sex. Mechanism of gastric absorptions describes that villus tips can take up particles of 5-150 µm, intestinal macrophages up to 1 µm and enterocytes are able to take 300-400 nm particles.[20, 21] This provides a better size window for gastric absorption of sub-micron particles. As drug need to be water soluble in order to get absorbed across the gastric barrier, lecithin based organic NCs were able to carry more drug into the blood stream. Plasma concentrations are shown in Figure 5 and pharmacokinetics parameters derived from this data are listed in Table 2. Results obtained indicate that maximum plasma concentrations (Cmax) for both AmB and water soluble injectable AmB-deoxycholate complex (Anfotericina FADA®) were 17 and 19 ng/ml which were far below than those achieved by Lec-AmB NCs. Highest plasma levels of AmB (165 ng/mL) were achieved in case of Lec-Amb Tw NPs. While in case of Lec-AmB PEG NPs and AmB Liposomes, the Cmax values were comparable but less than that of Lec-AmB Tw NPs.

Time for maximum plasma concentration (Tmax) was same i.e. 2.5 h for Tw and PEG Lec-AmB NPs but in case of AmB Liposomes it was 5 h. With an oral dose of just 4mg/kg, these oral bioavailability results are comparable to a few reported NCs for enhancing oral bioavailability of AmB.[8, 20, 22, 23]

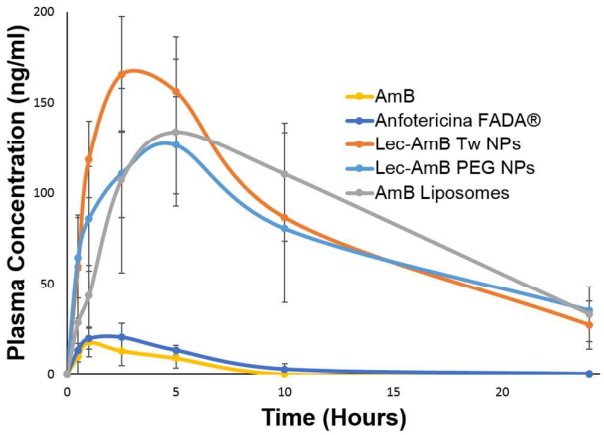

Fig. 5 Plasma concentrations of Amphotericin B dispersion in water, Amb Deoxycholate complex and Amb NCs, at different time intervals, after oral administration of 4mg/kg dose (n=4, mean±SD).

There is, however, no previous data for the oral bioavailability of water soluble deoxycholate complex of AmB. However, once absorbed, pharmacokinetics parameters were better for complex as compared to AmB itself, making it suitable for parenteral administration. Compared to AmB deoxycholate complex, better solubility and gastric absorption of Lec-AmB NPs resulted in increased oral bioavailability. This clearly shows the merit of biocompatible NCs over physical dispersion/complex or surfactant based solubilization approaches in order to improve oral pharmacokinetics of hydrophobic drugs.[24] Surface of NPs was modified with Tween and PEG to investigate the effect of surface engineering on solubility as well as gastric absorption and subsequent pharmacokinetics parameters. Mucoadhesion plays an important role in subsequent oral absorption of NPs and is affected by their nature, size, hydrodynamic radius and zeta potential. [25, 26]

Table 2. Pharmacokinetics Parameters for Lec-Ambithin NPs Cmax and Tmax, maximum plasma concentration and time taken to reach that maximum plasma concentration.

Pharmacokinetic Parameter	AmB	Anfotericina FADA®	Lec-AmB Tw NPs	Lec-AmB PEG NPs	Lec-AmB Liposomes
Dose (mg/kg)	4	4	4	4	4
C_{max} (ng/ml)	17.4 ± 7.8	19 ± 4.6	165.7 ± 31.7	126.4 ± 23.9	133.5 ± 40.4
T_{max} (h)	2.5	2.5	2.5	2.5	5
$AUC_{0\text{-}24h}$ (ng.hr/ml)	81 ± 44	141 ± 31	2078 ± 667	1824 ± 391	2052 ± 579
Cl (L hr^{-1} kg^{-1})	39.9 ± 7.4	29.5 ± 7.7	2.1 ± 0.7	2.3 ± 0.4	2.09 ± 0.6
Fr	1	1.5 ± 0.29	21.25 ± 5.3	18.97 ± 3.6	21.75 ± 7.2
MRT (h)	4.2 ± 1.4	2.7 ± 0.7	7.75 ± 1.2	9.09 ± 0.5	9.33 ± 0.6

$AUC_{0\text{-}24}$ h, area under curve from 0 to 24 h. Cl (clearance); Fr (relative bioavailability); MRT (mean residence time that average drug molecule spent in-vivo); The parameters of AUC, Cmax, Cl, Fr and MRT of nano-formulations were significantly improved ($P<0.05$) in comparison with AmB dispersion and AmB-Dsx solution in water, given orally at equivalent dose of 4mg/kg. However AmB and AmB-Deoxycholate were mutually insignificantly different ($P>0.05$).

Such mixed interaction with mucus gut wall is responsible for better drug absorption in bioavailability profile of Tween and PEG coated NPs and liposomes. Positively charged particles have more adhesion with mucin (negatively charged mucus covering of gastric epithelial cells) as compared to negatively charged particles. [25] Tween coated NPs showed relatively less negative zeta potential which may be responsible for better oral bioavailability (Table 3).

Table 3. Average zeta potential and hydrodynamic diameter of lecithin NCs for AmB (Mean ± SD)

Sample Name	Zeta Potential (mV)	Diameter (nm)
Lec-AmB PEG NPs	-55 ± 3	298 ± 28
Lec-AmB Tween NPs	-37 ± 2.8	209 ± 16
Lec-AmB Liposomes	-21 ± 1	103 ± 7

More negative zeta potential of PEG coated NPs may reduce their interaction with negatively charged mucin. This charge based mucorepulsive effect is further supported by greater hydrodynamic radius due to long PEG polymer chains. This tend to keep PEG coated particles entangled and entrapped in upper layers of mucus due to large size, hydrogen bonding and polymer entrapment.[27] In addition, large size of particles also resists their trancytosis. With increase in particle size, rate of absorption decreases which is another contributing reason for difference in bioavailability pattern of PEG and Tween coated particles.[28] Tween coated particles exhibited biphasic elimination, absent in other formulations, revealing the nanoparticle dependent excretion of drug from the body. This demonstrates the advantage of surface engineering of NCs to control their in-vivo fate in addition to improving their gastric absorption and bioavailability profile in body, after absorption.

The small size of liposomes should result in more oral absorption because small sized particles are usually able to squeeze through the mucin network but their highly negative zeta potential reduces their interactive adhesion with mucin and results in less oral absorption. It also explains the relative competitive status of mono vs bilayer nanostructures for oral absorption. Lecithin bilayer liposomes are slow to absorb (Tmax, 5 h) with lesser Cmax compared to Tween coated particles but they were able to retain the drug in systemic circulation for longer period of time i.e. slow rate of absorption and slow rate of elimination from the circulatory system. Obtained plasma levels correlate well with required therapeutic plasma levels of AmB i.e. 320 ng to 1.21µg/ml in systemic mycosis and about 1µg/ml in experimental models of visceral leishmania.[29, 30] Higher therapeutic plasma levels with lecithin NCs can be achieved by increasing lecithin concentration to enhance the entrapment of drug. Enhanced oral bioavailability of AmB entrapped in lecithin NCs may be due the protection of drug from acidic degradation in stomach and subsequent efflux via P-glycoproteins, which is the case if AmB is ingested in free form. This shows the potential use of lecithin NCs for oral delivery of proteins and peptides. Lecithin-AmB NCs were also evaluated for antileishmanial activity and the results are shown in Figure 6.

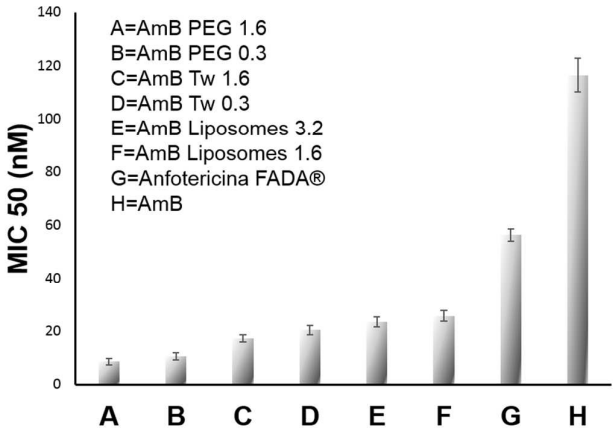

Fig. 6 Antileishmanial activity of Amphotericin B nano-formulations in terms of minimum inhibition concentration. Each formulation is evaluated for its two different size/encapsulation efficiency samples i.e. 1.6 and 0.3 mg/ml for Tween and PEG while 3.2 and 1.6 mg/ml for liposomes with insignificant difference in between them ($P>0.05$) but difference in nano-formulation and standard i.e. water soluble AmB-deoxycholate complex is statistically significant ($P<0.05$).

Biological activity of PEG coated NPs was far more than other NCs or free form of drug. Structural features of leishmanial parasite reveals layer of hydrophilic phospholipoglycans which not only protect it from immune response but also keep it safe inside macrophages i.e. amastigote form. Pharmacological target of

Amphotericin B in leishmania is ergosterol in cell membrane. Reduced activity of drug against parasite is attributed to phospholipoglycans coat on membrane and slow efflux phenomenon which is present even in sensitive strain.[31] Thus activity of these hydrophilic surfactant coated NCs may be attributed to their ability to interact with the outer coat of parasite and deliver the drug. Leishmanial parasite cell is large enough (15-30 µm in length and about 5 µm in width) to provide a plenty of space for cell-particle interaction. PEG coated NPs showed more favorable results as compared to Tween coated NPs, probably because of better particle-parasite interaction. As discussed earlier, the small particles showed greater anti-parasitic activity. In case of liposomes, similar trend was observed but overall activity was decreased due to the absence of surfactants on surface to enhance nanocarriers-parasite interaction. Recently reported PLGA (Poly lactide co-glycolic acid) NPs of AmB are far better than conventional AmB formulations i.e. Ambisomes® and Fungizone®.[32] However lecithin based NPs, coated with PEG, showed 6.3 times reduction in IC 50 of AmB which is much superior then that achieved by PLGA NP. The improvement in IC 50 by Lec-AmB Tw and liposomes was improved by only 3 and 2 times respectively, as compared to control i.e. AmB dispersion in water.

Conclusions

Synthesis of lecithin based NCs comprised of non-toxic, biocompatible and biodegradable constituents, is demonstrated to improve the pharmacological and oral pharmacokinetic profile/in-vivo fate of Amphotericin B. Coating these NCs with PEG and Tween showed even more promising results. PEG functionalized NPs showed more promising antileishmanial activity among these NCs that may be because of their better interaction with the biological systems and membranes. Tween coated lecithin-drug NPs showed better mucus penetrating properties as revealed in oral bioavailability and pharmacokinetic results. On the other hand, lecithin liposomes were though expected to carry more drug and better biological activity but they were not that effective to deliver the drug inside parasitic cells. In oral bioavailability profile, however, lecithin liposomes showed sustained release of drug inside blood stream that was not observed in other formulations. In previous studies, lecithin is reported to be used as a part of pharmaceutical formulations but in very small concentrations along with other synthetic polymeric materials. These promising results encourage the use of food grade materials as biocompatible nanocarriers for drug delivery owing to their potential to improve the pharmacological/ pharmacokinetic profile and providing a valuable tool to overcome physicochemical barriers associated with drug molecules.

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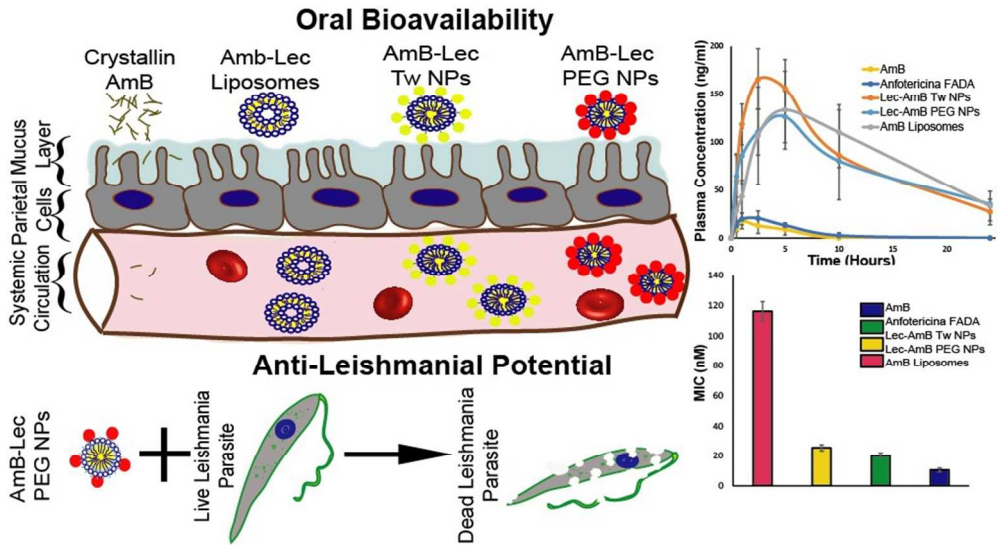
Notes and references

1. D. W. Bates, L. Su, T. Y. Donghui, G. M. Chertow, D. L. Seger, D. R. Gomes and R. Platt, *Kidney international*, 2001, **60**, 1452-1459.
2. M. S. Saag, W. G. Powderly, G. A. Cloud, P. Robinson, M. H. Grieco, P. K. Sharkey, S. E. Thompson, A. M. Sugar, C. U. Tuazon and J. F. Fisher, *New England Journal of Medicine*, 1992, **326**, 83-89.
3. M. Grogl, T. N. Thomason and E. D. Franke, *The American journal of tropical medicine and hygiene*, 1992, **47**, 117-126.
4. E. M. de Moraes Flores, E. P. dos Santos, J. S. Barin, R. Zanella, V. L. Dressler and C. F. Bittencourt, *Journal of Analytical Atomic Spectrometry*, 2002, **17**, 819-823.
5. J. N. Sangshetti, F. A. Kalam Khan, A. A. Kulkarni, R. Arote and R. H. Patil, *RSC Advances*, 2015, **5**, 32376-32415.
6. T. Takagi, C. Ramachandran, M. Bermejo, S. Yamashita, L. X. Yu and G. L. Amidon, *Molecular Pharmaceutics*, 2006, **3**, 631-643.
7. D. R. Serrano, A. Lalatsa, M. A. Dea-Ayuela, P. E. Bilbao-Ramos, N. L. Garrett, J. Moger, J. Guarro, J. Capilla, M. P. Ballesteros and A. G. Schatzlein, *Molecular pharmaceutics*, 2015, **12**, 420-431.
8. J. Italia, M. Yahya, D. Singh and M. R. Kumar, *Pharmaceutical research*, 2009, **26**, 1324-1331.
9. J. P. Jain, M. Jatana, A. Chakrabarti and N. Kumar, *Molecular pharmaceutics*, 2010, **8**, 204-212.
10. N. Yanasarn, B. R. Sloat and Z. Cui, *International journal of pharmaceutics*, 2009, **379**, 174-180.
11. E. Arab Tehrany, C. J. Kahn, C. Baravian, B. Maherani, N. Belhaj, X. Wang and M. Linder, *Colloids and Surfaces B: Biointerfaces*, 2012, **95**, 75-81.
12. J. Sykora, S. Yilma, W. C. Neely and V. Vodyanoy, *Langmuir*, 2003, **19**, 858-864.
13. E. Directive, *Official Journal of the European Union*, 2010, **276**, 33-74.
14. L. Shargel, B. Andrew and S. Wu-Pong, *Applied biopharmaceutics & pharmacokinetics*, McGraw-Hill New York:, 2005.
15. I. Bekersky, R. M. Fielding, D. E. Dressler, J. W. Lee, D. N. Buell and T. J. Walsh, *Antimicrobial agents and chemotherapy*, 2002, **46**, 828-833.
16. S. Nabi, N. Ahmed, M. J. Khan, Z. Bazai, M. Yasinzai and Y. Al-Kahraman, *World Applied Sciences Journal*, 2012, **19**, 1495-1500.
17. R. Angelico, A. Ceglie, G. Colafemmina, F. Delfine, U. Olsson and G. Palazzo, *Langmuir*, 2004, **20**, 619-631.
18. U. S. F. a. D. Administration, *The GRAS Substances (SCOGS) Database*.
19. M. Gagoś and M. Arczewska, *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 2010, **1798**, 2124-2130.
20. S. Jain, P. U. Valvi, N. K. Swarnakar and K. Thanki, *Molecular pharmaceutics*, 2012, **9**, 2542-2553.
21. D. O'hagan, *Journal of anatomy*, 1996, **189**, 477.
22. P. Gershkovich, E. K. Wasan, M. Lin, O. Sivak, C. G. Leon, J. G. Clement and K. M. Wasan, *Journal of antimicrobial chemotherapy*, 2009, **64**, 101-108.
23. Z. Yang, Y. Tan, M. Chen, L. Dian, Z. Shan, X. Peng and C. Wu, *AAPS PharmSciTech*, 2012, **13**, 1483-1491.
24. T. Vasconcelos, B. Sarmento and P. Costa, *Drug discovery today*, 2007, **12**, 1068-1075.

25. H. Takeuchi, Y. Matsui, H. Yamamoto and Y. Kawashima, *Journal of controlled release*, 2003, **86**, 235-242.
26. H. Takeuchi, H. Yamamoto and Y. Kawashima, *Advanced Drug Delivery Reviews*, 2001, **47**, 39-54.
27. S. K. Lai, Y.-Y. Wang and J. Hanes, *Advanced drug delivery reviews*, 2009, **61**, 158-171.
28. A. T. Florence, *Drug discovery today: technologies*, 2005, **2**, 75-81.
29. B. T. Fields, J. H. Bates and R. S. Abernathy, *Applied microbiology*, 1970, **19**, 955-959.
30. J. Sanchez-Brunete, M. Dea, S. Rama, F. Bolás, J. Alunda, R. Raposo, M. Méndez, S. Torrado-Santiago and J. Torrado, *Antimicrobial agents and chemotherapy*, 2004, **48**, 3246-3252.
31. N. Mbongo, P. M. Loiseau, M. A. Billion and M. Robert-Gero, *Antimicrobial agents and chemotherapy*, 1998, **42**, 352-357.
32. H. Van de Ven, C. Paulussen, P. Feijens, A. Matheeussen, P. Rombaut, P. Kayaert, G. Van den Mooter, W. Weyenberg, P. Cos and L. Maes, *Journal of Controlled Release*, 2012, **161**, 795-803.



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