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Design and In-silico Study of Hydroxychloroquine and Ivermectin Malaria-Based Drug Delivery System using Liposomal and Missile Carriers

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Abstract

Malaria remains a major global infectious disease, contributing significantly to morbidity and mortality. Although various antimalarial drugs are available, their clinical application is often limited by severe side effects. Targeted drug delivery systems have been explored as a strategy to enhance therapeutic efficacy and minimize systemic toxicity. In this in-silico study, a liver-targeted drug delivery system for hydroxychloroquine and ivermectin was designed and modeled using liposomal carriers (dipalmitoyl phosphatidylcholine, DPPC) and micellar systems. Molecular dynamics simulations were conducted using GROMACS 2022.2 with the MARTINI coarse-grained force field. DPPC liposomes and polymeric micelles were modeled to assess drug encapsulation and delivery efficiency. Structural and dynamic properties, including moment of inertia (MOI), solvent-accessible surface area (SASA), and radial distribution function (RDF), were analyzed at various simulation stages. Supplementary laboratory validation was performed, involving liposome preparation, size characterization, encapsulation efficiency, and release kinetics. Simulations revealed that DPPC liposomes were highly effective for delivering hydroxychloroquine to the lysosomal compartment, while micelles were found to enhance ivermectin solubility in hydrophilic environments. In vivo validation was not conducted, representing a key limitation. Nevertheless, valuable insights into the potential of liposomal and micellar carriers for targeted malaria therapy were provided. Further experimental studies are recommended to validate and refine these computational findings.

Keywords: Hydroxychloroquine, Ivermectin, liposomes, micelles, Molecular dynamics

Introduction

Significant contributions to global disability and mortality are made by liver diseases, particularly those associated with malaria, the most prevalent vector-borne parasitic infection worldwide (1). Transmission of malaria occurs through bites from female Anopheles mosquitoes, caused by protozoan parasites of the Plasmodium genus, including *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* (2). Variations in malaria epidemiology are observed across geographical regions, with differences in incidence, causative species, severity, antimicrobial resistance, and mortality patterns (3). Despite advancements in treatment, over 400,000 lives are claimed annually by malaria, with nearly two-thirds of deaths occurring in children under five years of age. Addressing malaria-related liver complications remains a critical challenge in pharmaceutical and bio-

medical research (4,5).

The rising incidence of drug-resistant malaria has been recognized as a significant obstacle to global eradication efforts (6). Given the liver's role as the largest reticuloendothelial organ, hepatic macrophages (Kupffer cells) have been identified as promising targets for therapeutic interventions in malaria-associated liver pathology (7,8). Liver-specific drug delivery has been shown to reduce systemic side effects, limit off-target drug distribution, and enhance therapeutic efficacy through increased drug accumulation in target cells (9). However, physiological barriers such as reticuloendothelial system (RES) uptake, opsonization, and first-pass metabolism have been noted to complicate conventional therapeutic approaches. These challenges underscore the need for novel strategies to manage advanced liver-stage malaria infections effectively. Resistance to antimalarial drugs is observed when Plasmodium parasites survive and replicate despite exposure to drug concentrations typically sufficient to inhibit or eliminate them, further complicating treatment (10).

Although Plasmodium parasites are effectively eliminated by antimalarial drugs, their clinical use is frequently restricted by severe side effects (11). Extensive investigations have been conducted on hydroxychloroquine and ivermectin for their activity against malaria and various viral infections. Hydroxychloroquine's effect is exerted primarily through the creation of an acidic environment that disrupts intracellular processes, while ivermectin's inhibition of viral replication is achieved by disrupting importin-mediated nuclear transport (12–15).

The modification of drug pharmacokinetics and pharmacodynamics through advanced delivery systems has been increasingly emphasized as a promising strategy for improving therapeutic outcomes (16). Liposomes, artificial spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core, have been widely studied as lipid-based nanocarriers. Their capability to encapsulate both hydrophilic and hydrophobic drugs has been recognized as advantageous for enhancing the therapeutic efficacy of antimalarial agents. Specifically, the bioavailability and controlled release of hydroxychloroquine for malaria treatment have been shown to be improved by liposomal systems (17).

Micellar systems, formed through the self-assembly of surfactant or polymer molecules, have also been recognized for their prominence in drug delivery research. Greater stability compared to liposomes has been noted, making micelles particularly effective for improving the solubility and delivery of poorly water-soluble drugs such as ivermectin (18, 19).

In this study, a liver-targeted drug delivery system for hydroxychloroquine and ivermectin was developed using liposomal and micellar carriers. Sustained release properties and entrapment efficiency were evaluated through an in-silico approach, providing insights into the potential of nanocarrier-based systems for safer and more effective antimalarial therapy.

Materials and Methods

In-silico analysis

Molecular dynamics (MD) simulations were performed using GROMACS version 2022.2 with the MARTINI coarse-grained force field (version 3). Two nanocarrier systems, liposomes and micelles, were modeled to evaluate their potential as targeted delivery vehicles for hydroxychloroquine and ivermectin (20-22).

Simulation of ivermectin and hydroxychloroquine with DPPC liposomes

Liposomes, lipid-based nanocarriers consisting of artificial spherical vesicles with one or more phospholipid bilayers surrounding an aqueous core, have been recognized for their potential to enhance the therapeutic performance of drugs against Plasmodium infections. Dipalmitoylphosphatidylcholine (DPPC) was selected as a well-characterized lipid for liposome formulation. The liposomal carrier DPPC877 (12 beads) was utilized as a passive

targeting system for the delivery of hydroxychloroquine and ivermectin (Fig. 1a, b).

A simulation box was constructed, and the liposomal carrier was assembled. Ivermectin (300, 100, or 50 molecules) and hydroxychloroquine (50 molecules) were individually incorporated into the system. Simulations were conducted for 1 μs with a time step of 20 fs. System stability and drug-carrier interactions were analyzed post-simulation using moment of inertia (MOI), solvent-accessible surface area (SASA), and radial distribution function (RDF) (23,24).

Stabilization of ivermectin-loaded liposomes

Cholesterol hemisuccinate was incorporated into the bilayer to enhance liposome stability, reducing fragility and improving resistance to environmental stress (Fig. 1e). System stability was assessed through SASA and total energy measurements. A pH-sensitive component was introduced to enable controlled release at acidic pH, as ivermectin requires lysosomal release. Simulations were performed under physiological conditions: 310 K (37 °C), 1 atm pressure, and pH 5, mimicking the lysosomal environment (25,26).

Micellar carrier simulations

Palmitoyl phosphatidylcholine (PPC) monomers, derived from DPPC fatty acids, were used to construct micelles in aqueous solution, capable of solubilizing hydrophobic drugs such as ivermectin. Structural analysis was conducted, including calculation of the radius of gyration for core and shell regions over simulation time, using the following formulas:

$$R_{gyrate}-PPC = 1.9675 \Rightarrow RPPC = 1.9675 \times \sqrt{5}\,3 = 2.5400nm$$

$$R_{gyrate}-PPC_{-tail} = 1.4134 \Rightarrow RPPC = 1.4134 \times \sqrt{5}\,3 = 1.8247nm$$

$$R_{gyrate}-PPC_{-head} = 2.5400 - 1.8247 = 0.7153n$$

The coarse-grained structure of PPC monomers is presented in Figure 1d (27-29).

Supplementary laboratory validation

Liposomes were prepared using the thin-film hydration method. L-α phosphatidylcholine (5 mg) and cholesterol (2.5 mg) were dissolved in 5 mL of chloroform within a 50 mL round-bottom flask for 20 minutes. The solvent was removed by rotary evaporation under vacuum for 1 h at 60 °C, resulting in a dried lipid film. The film was hydrated with 5 mL distilled water at 65 °C, yielding multilamellar vesicles (MLVs), which were sonicated with a probe ultrasonicator at 300 W for 5 min. For ivermectin-loaded liposomes, ivermectin was dissolved in chloroform before evaporation (24).

Particle size was measured using a HORIBA SZ-100 particle sizer, and encapsulation efficiency (EE) was determined. Varying concentrations of ivermectin (1, 2, 5, and 10 mg) were encapsulated, and liposomes were centrifuged at 5000 g for 10 min to remove free drug. Triton X-100 was used to lyse liposomes, releasing encapsulated ivermectin into the buffer (30).

Drug concentration was quantified using an Azura Knauer HPLC system with a C18 column and UV detection at 245 nm, employing a mobile phase of water (5%) / methanol (95%) at a flow rate of 1 mL/min (31,32).

Drug release kinetics were investigated at 4 °C, 25 °C, and 37 °C. Ivermectin-loaded liposomes (10 mL) were placed in 20 mL PBS buffer, with 1 mL aliquots distributed into 30 tubes. Ten tubes were incubated at each temperature. At intervals of 0.5, 1, 2, 4, 6, 8, 16, 24, 36, and 48 h, one tube was centrifuged at 4 °C, 5000 g for 10 min, and released ivermectin was quantified by HPLC. Concentration-time profiles were plotted to evaluate release behavior (33).

Results and Discussion

Design of liposomal and micellar carriers and stabilization

Coarse-grained models of phosphatidylcholine systems were constructed to gener-

ate (i) DPPC liposomes and (ii) PPC micelles as drug carriers (Fig. 1a, b). DPPC877 (12 beads per lipid) was employed as a passive carrier for ivermectin and hydroxychloroquine (Fig. 1a, c, d). Cholesterol hemisuccinate (CHEMS) was incorporated to enhance bilayer robustness under neutral conditions and enable pH-triggered release (Fig. 1e). PPC monomers (54 units) were assembled into micelles in water (Fig. 1b).

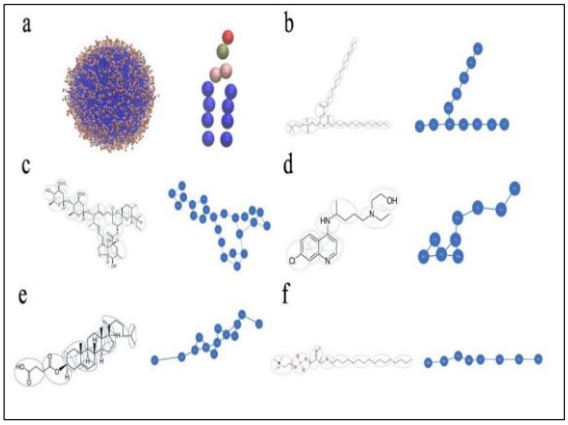

Figure 1. (a) Coarse-grained design of phosphatidylcholine and its formation as a liposome. (b) Coarse-grained modeling of phosphatidylcholine, (c) Ivermectin, (d) Hydroxychloroquine, (e) Cholesteryl hemisuccinate, and (f) Lyso phosphatidylcholine.

Ivermectin and Hydroxychloroquine in DPPC liposomes

Simulations were initiated with ivermectin placed around a preformed DPPC liposome (1 μs, Δt = 20 fs). At high loading (300 molecules), rapid coating of the bilayer surface by ivermectin was observed, leading to shape fluctuations and carrier destabilization (Fig. S1). Smooth MOI traces were recorded up to 50 ns, followed by large excursions consistent with transitions from near spherical to ellipsoidal/cylindrical morphologies, indicating reduced stability (Fig. S2a). SASA analysis revealed a rapid decrease in ivermectin alone to ~7 ns, plateauing thereafter, indicating aggregation in water. Profiles at 50 ns (drug only) and 500 ns (drug + liposome) are shown in Fig. S2b and c. Stability was improved by reducing the load to 100 and 50 molecules (Fig. S2d and e), with the 50-molecule system maintaining liposome integrity (Fig. 2a).

Radial distribution function (RDF) profiles indicated water density at the box center, hydrophilic headgroups adjacent to the aqueous phase, and hydrophobic tails defining the membrane interior (Fig. 2b). Overlap of ivermectin density with the hydrophobic core was observed, confirming preferential partitioning into the bilayer.

Water was detected in the center of the box up to 2 nm, with hydrophilic heads positioned from 2 to 5 nm and 6 to 9 nm. The hydrophobic tail was located 3 nm from the center, extending to 8 nm, with a hydrophobic diameter of 5 nm and a total carrier diameter of 7 nm. Ivermectin (red) was observed within the hydrophobic part of the carrier (blue), with water dispersion decreasing toward the center to nearly zero (Fig. 2b).

Simulations with 50 hydroxychloroquine molecules demonstrated greater carrier stability compared to ivermectin. Initial fluctuations in MOI were observed as drug molecules associated with the membrane, followed by stabilization as the system relaxed (Fig. 2c). Reduced water contact for DPPC headgroups in the presence of hydroxychloroquine was indicated by SASA profiles, while the drug retained hydration, consistent with surface/headgroup association rather than deep insertion (Fig. S3a, b,c). A steep decrease in system potential energy was observed initially, followed by a gradual decline, indicating sustained stabilization (Fig. S3d).

Predominant interactions with polar headgroups and minimal penetration into the hydrophobic interior were shown by RDF, with low water density within the bilayer midplane (Fig. 2d).

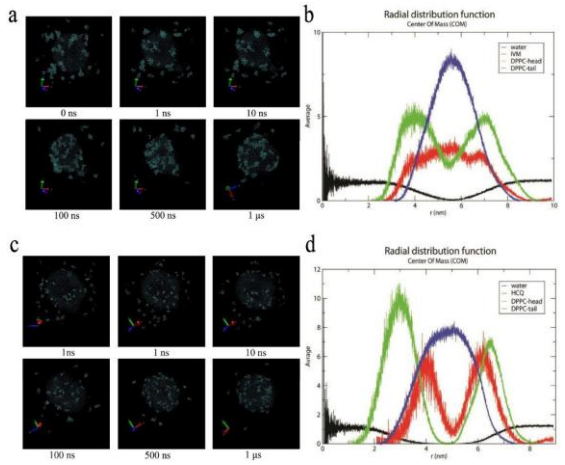

Figure 2. (a) Evolution of DPPC liposome simulation with 50 ivermectin drugs. **(b)** Radial distribution function for the DPPC carrier with 50 ivermectin drugs: water in black, ivermectin in red, hydrophilic heads (choline, phosphate, glycerol) in blue, hydrophobic tails in green. **(c)** Evolution of liposome simulation with 50 hydroxychloroquine drugs, with most drugs stabilized at the end. **(d)** Radial distribution function of the carrier and hydroxychloroquine: water in black, hydroxychloroquine in red, hydrophilic ends in green, hydrophobic end in blue.

Ivermectin with stabilized, pH-responsive liposomes

Membrane cohesion at neutral pH was increased, and pH sensitivity was conferred by incorporating CHEMS. A decrease in SASA of hydrophobic regions was observed post-insertion, while CHEMS–water SASA remained largely unchanged (Fig. S4a). A monotonic decline in total potential energy was recorded (Fig. S4b). No abrupt morphological transitions were indicated by MOI traces, and CHEMS was placed alongside phospholipids within the bilayer by mass–radius distributions.

Ivermectin in PPC micelles

Given the liposomal instability at high ivermectin loads, PPC micelles were evaluated. PPC monomers (derived from DPPC fatty acid tails) self-assembled into micelles that solubilized hydrophobic cargo (Fig. 1f; Fig. 3a). SASA during assembly is shown in Fig. S5a. The radius of gyration (Rg) decreased over time and stabilized by ~28 ns, coincident with micelle formation (Fig. S5b). Analytical values used in the model were:

$$Rgyrate{-}PPC = 1.9675 \Rightarrow RPPC = 1.9675 \times \sqrt{5/3} = 2.5400n$$

$$Rgyrate{-}PPC_{-tail} = 1.4134 \Rightarrow RPPC = 1.4134 \times \sqrt{5/3} = 1.8247n$$

$$Rgyrate{-}PPC_{-head} = 2.5400 - 1.8247 = 0.7153n$$

RDF confirmed ivermectin enrichment in the micellar hydrophobic core with limited water penetration (Fig. 3b, S4c), supporting improved aqueous solubility relative to liposomes.

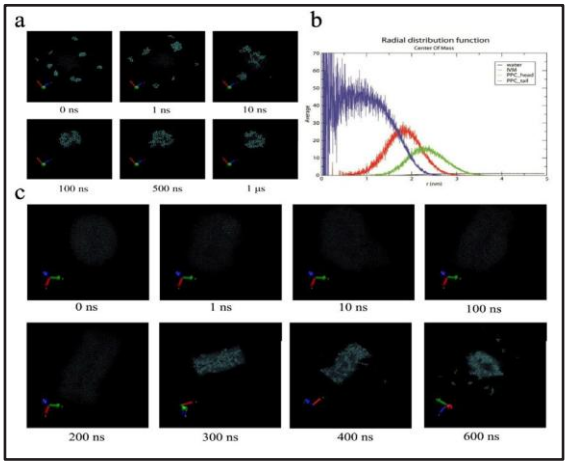

Figure 3. (a) Evaluation of PPC micelle in the presence of Ivermectin (b) Radial distribution function of PPC-Ivermectin micelle. (c) The evolution of the DPPC system, Hydroxychloroquine, and cholesterol hemisuccinate at pH 5.

Simulation of DPPC Carrier with Hydroxychloroquine under Lysosomal pH

Molecular dynamics simulations were conducted to evaluate the behavior of the DPPC liposomal system containing hydroxychloroquine and the pH-sensitive stabilizing agent, cholesterol hemisuccinate, under lysosomal

conditions (pH 5). As illustrated in Figure 3c, the carrier initially exhibited uncoordinated molecular motion, followed by progressive structural transitions. Within the first ns, the spherical morphology of the system began to deform, gradually evolving into an elliptical configuration by 100 ns. At 200 ns, the liposomal structure was further reorganized into a rod-like morphology. From this point, hydroxychloroquine molecules, highlighted for visualization, gradually detached from the carrier. Drug release became evident after approximately 300 ns, with substantial release observed between 400-600 ns, at which time more than half of the encapsulated molecules were liberated into the surrounding environment.

Laboratory Validation of Liposomal Carrier Preparation

Encapsulation efficiency of ivermectin in DPPC liposomes was evaluated at different initial drug concentrations (0.2-2 mg/mL). As shown in Figures 4 b and c, the absolute amount of encapsulated drug increased with higher initial concentrations, whereas the percentage of encapsulation efficiency decreased. The relative encapsulation efficiencies at 0.2, 0.5, 1, and 2 mg/mL were 7.08%, 4.35%, 1.87%, and 1.08%, respectively.

DLS analysis using a HORIBA SZ-100 particle sizer at 25 °C revealed a mean hydrodynamic diameter of 290.9 ± 9.8 nm with a polydispersity index (PDI) of 0.198 (Fig. 4a). Zeta potential measurements confirmed stable liposomal formulations. Drug release profiles at 4, 25, and 37 °C are shown in Figure 4d. Release kinetics were temperature dependent, with significantly accelerated release at higher temperatures. At 37 °C, equilibrium drug release was reached in less than 2 h, compared to 6 h at 25 °C and 24 h at 4 °C.

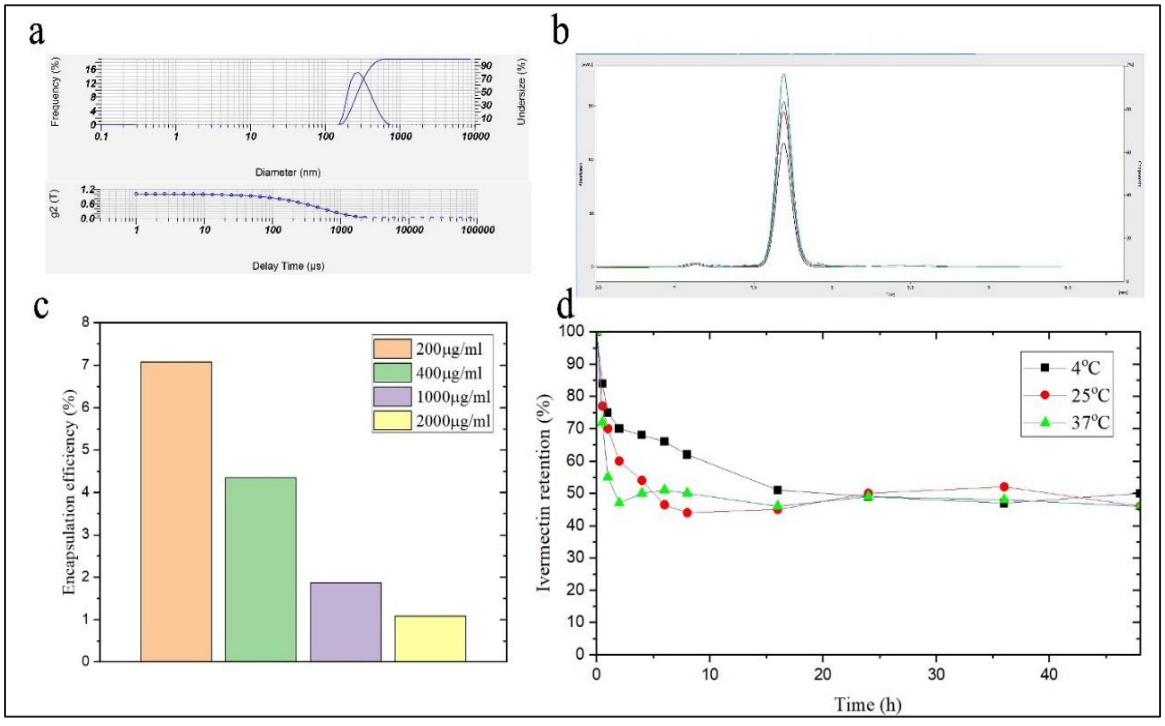

Figure 4. (a) Hydrodynamic radius of DPPC liposome. (b) HPLC diagram of the Encapsulated drug. (c) Encapsulation efficiency of Ivermectin in DPPC liposome. (d) Ivermectin release kinetics at various temperatures.

Discussion

This study employed molecular dynamics simulations and experimental validation to investigate DPPC liposomes and PPC micelles as nanocarriers for hydroxychloroquine and ivermectin, two antimalarial agents with known therapeutic limitations. Our findings demonstrated that DPPC liposomes provided a stable environment for hydroxychloroquine, favoring association with polar headgroups rather than deep penetration into the bilayer. In contrast, ivermectin induced significant liposomal destabilization at high concentrations, while PPC micelles offered improved solubility and structural stability.

Liposomal carriers have long been recognized as effective vehicles for hydrophilic and amphiphilic drugs, owing to their biocompatibility, capacity for controlled release, and ability to accumulate in target tissues via passive or active targeting strategies (34). Our simulations support these established properties by showing that hydroxychloroquine preferentially associates with DPPC headgroups, maintaining liposome integrity. Previous studies similarly reported that liposomal encapsulation of antimalarial drugs enhances their pharmacokinetic profiles and reduces systemic toxicity (35-37).

For ivermectin, micellar carriers proved more advantageous. Micelles are well-suited to solubilize hydrophobic molecules, improving their aqueous dispersibility and therapeutic efficacy (38, 39). Our RDF analysis confirmed ivermectin localization within the micellar hydrophobic core, consistent with prior reports on polymeric micelles enhancing the bioavailability of poorly soluble drugs. This observation is clinically relevant, as ivermectin's limited solubility and systemic toxicity remain obstacles for repurposing in malaria therapy (20, 40, 41).

The laboratory validation corroborated *in silico* predictions. Encapsulation efficiency decreased with increasing ivermectin concentration, consistent with saturation effects reported in previous liposomal systems (42). The observed temperature-dependent release profiles align with known thermodynamic principles of lipid bilayers, where increased fluidity at higher temperatures accelerates drug diffusion. Second-generation liposomes, i.e., stimuli-responsive liposomes, have the potential to not only provide site-specific chemotherapy, but also trigger drug release and thus greater spatial and temporal control of therapy. Temperature-sensitive liposomes are an especially attractive option, as tumors can be heated in a controlled and predictable manner with external energy sources. Traditional thermosensitive liposomes are composed of lipids that undergo a gel-to-liquid phase transition at several degrees above physiological temperature. More recently, temperature sensitization of liposomes has been demonstrated with the use of lysolipids and synthetic temperature-sensitive polymers (43).

A formulation screen of 18 ivermectin liposomes (DOPC/SPC/DSPC; film hydration or ethanol injection) was compared, reporting stable colloids (~200 nm, PDI≈0.39, ζ≈-40 mV), high encapsulation (>80%; ~4% loading), and improved antiviral IC50 versus free drug (44). In the present system, destabilization of DPPC bilayers by ivermectin at higher drug loads was observed, and encapsulation efficiency declined with concentration, whereas PPC micelles maintained solubilization and structural integrity. These data suggest that ivermectin performance is strongly dependent on composition and method: DPPC-rich bilayers were vulnerable, but alternative lipids yielded stable, bioactive liposomes, while micelles were advantageous for higher drug loading (44).

In support of our findings, a recent study showed that ivermectin encapsulated in SPC/DOPC/cholesterol/DCP liposomes synthetized by ethanol injection method, achieved markedly higher cellular uptake in Vero E6 cells (≈13–60%) than free drug (≈2%) while shifting cytotoxicity from a CC50 of ~10 μM (free) to >110 μM (liposomal). These data indicate that rational tuning of lipid composition can en-

hance ivermectin delivery and mitigate toxicity, potentially addressing the destabilization we observed with DPPC-rich formulations (45). Together with our results favoring micellar solubilization at higher drug loads, this work suggests that liposomal routes remain viable when membrane composition and drug-to-lipid ratios are optimized (45).

A key limitation of the current study is the absence *in vivo* validation. While simulations and in vitro assays provide mechanistic insights, in vivo studies it is essential to assess biodistribution, pharmacokinetics, and immune interactions. Moreover, future research should explore ligand-functionalized liposomes for liver-targeted delivery to further improve therapeutic specificity and minimize systemic exposure.

Conclusion

The efficacy of DPPC liposomes for hydroxychloroquine delivery and PPC micelles for ivermectin solubilization was demonstrated. Computational modeling combined with laboratory validation provided evidence supporting the potential of nanocarrier-based strategies to enhance the safety and efficacy of antimalarial agents. In vivo studies are recommended to further validate these findings for clinical translation.

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Author contributions

All authors conceived the presented idea, carried out the experiment, and wrote the manuscript equally.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Not applicable

Funding

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Data availability statement

All data will be presented according to the reviewer's requirement

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