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Research Article

Assessment of ex-vivo intestinal permeability and lymphatic uptake of curcumin and piperine-loaded nanostructured lipid carriers

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Abstract

Curcumin (CUR) is a naturally occurring compound in food known for its potential pharmacological activity but faces challenges due to its high metabolism. Piperine (PIP) is an effective inhibitor of metabolizing enzymes, enhancing the bioavailability of CUR. This work evaluated the lymphatic absorption and *ex-vivo* intestinal permeability of nanostructured lipid carriers (NLCs) containing PIP and CUR (CP NLCs). The optimized lipid formulation underwent *in-vitro*drug release, *ex-vivo* permeation, and lymphatic uptake studies utilizing chicken intestinal (jejunum) segments. Studies were conducted under different conditions, specifically in the presence and absence of the lymphatic uptake blocker Pluronic-F68 (PF68). PF68 is a non-ionic surfactant commonly used in pharmaceutical research, and it's known for its ability to inhibit lymphatic uptake. *In-vitro* drug release profiles indicated the controlled release of CUR and PIP from NLCs over 24 h. The *ex-vivo* permeability study demonstrated that CP NLCs exhibited higher permeation compared to CUR and PIP Suspension (CP Suspension). Studies on the lymphatic uptake of CP NLCs, conducted with and without the presence of the lymphatic uptake blocker PF68, demonstrated a decrease in drug permeation. However, in the absence of the lymphatic blocker, drug transport via the lymphatic path increased significantly by 4.07-fold for CUR and 6.56-fold for PIP. This means that the NLCs significantly enhanced the lymphatic transport of both CUR and PIP. The results imply that the lipid-based NLC system shows potential as a drug delivery method, improving solubility, and aiding in the lymphatic transport of both CUR and PIP.

Keywords: Curcumin; piperine; nanostructured lipid carriers; lymphatic uptake; payer's patches

Introduction

CUR, a hydrophobic polyphenol with significant therapeutic potential, faces practical challenges that limit its application. These challenges include poor aqueous solubility, swift systemic clearance, metabolism (both intestinal and hepatic), absence of cell targeting, and multidrug resistance despite its proven effectiveness and safety against various cancers [1]. However, when administered together, PIP and CUR co-administration has been reported to increase CUR bioavailability by 200% in human volunteers dramatically. This substantial enhancement is attributed to PIP's role in inhibiting the glucuronidation of CUR, thereby preventing its rapid metabolism and clearance.

Additionally, a similar dosage administered to healthy human volunteers resulted in a remarkable 2000% increase in CUR bioavailability [2,3]. The research started with formulating a combination of CUR and PIP to improve the oral bioavailability of CUR. Various formulation strategies were found during the literature survey, including sodium alginate nanoparticles [4], cubosomes [5], liquid self-nanoemulsifying drug delivery systems [6-8], solid zein-chitosan nanoparticles filled in capsules [9], liposomes [10], immediate-release tablets [11], solid dispersion amorphous powder [12], Solid self-nano emulsifying drug delivery system powder filled in capsules [13], internalizing argineglycine-aspartic

acid liposomes [14], unformulated actives [15], and ethosomes [16]. There was no published literature on CUR and PIP lipid-based matrix systems. Hence, a lipid-based approach was selected for development. Lipid formulations, which include a combination of excipients such as pure triglyceride oils, mixed glycerides, lipophilic surfactants, hydrophilic surfactants, and water-soluble co-solvents, present a promising solution. These formulations aim to improve bioavailability through lymphatic uptake, thereby reducing the need for large doses to achieve suitable serum concentrations. Various mechanisms for NLC disposition inside the body have been proposed via their selective uptake through lacteals or payer's patches. The NLC systems increase absorption from the gastrointestinal tract by accelerating the dissolution process, facilitating the formation of solubilized phases by reduction of particle size to the molecular level, yielding a solid-state solution within the carrier, changing drug uptake, efflux and disposition by altering enterocyte-based transport, and enhancing drug transport to the systemic circulation via the intestinal lymphatic system. During gastrointestinal tract passage, NLCs circumventing the digestion process can be either conveyed to the portal blood via paracellular route bypassing metabolism due to enterocyte enzymes or can be captured by M cells of payer's patches delivering NLCs to the lymphatic system [17-19]. Further, no study has been reported to understand the intestinal permeability and lymphatic uptake of CUR and PIP NLCs. Hence, the main objective of this study was to examine the intestinal permeability and lymphatic uptake of lipid-based nanoparticles to enhance the oral bioavailability of both CUR and PIP. The importance of these objectives cannot be overstated, as they could lead to significant advancements in drug delivery.

Materials and methods

Materials

The CUR gift sample was sourced from VAV Life Sciences, Mumbai, while PIP was procured from Sigma-Aldrich, India. Gift samples of Precirol ATO 5 (PRE), Labrafac Lipophile WL 1349 (LAF), and Gelucire 50/13 (G50/13) were obtained from Gattefosse, India. Additionally, gift samples of Tween 80 (T80), Polaxomer 188 (P188), also known as Pluronic F68 (PF68), and Polaxomer 407 (P407) were acquired from BASF, India. All chemicals used in this study, except for the specified gift samples, were of analytical reagent grade, ensuring their high quality and reliability. They were employed without additional purification, maintaining their integrity. The preparation of all solutions was conducted using Millipore (ultrapure) water, further enhancing the quality of our research.

Nanostructured lipid carriers (NLCs) formulation

The CP NLCs were formulated with the following components: CUR (0.08% w/v), PIP (0.04% w/v), PRE (3% w/v), LAF (2% w/v), T80 (0.125%), G50/13 (0.125%), and purified water. The preparation method utilized a modified hot melt emulsification process. The resulting formulation exhibited a particle size of 248.5 ± 12.8 nm, with a Polydispersity Index of 0.216 ± 0.021. The drug content was 99.70 ± 0.21 % and 100.36 ± 0.12% for CUR and PIP, respectively. The entrapment efficiency of CP NLCs was 99.80 ± 0.21% and 100.05 ± 0.07% for CUR and PIP respectively [20].

In-vitro drug release study

In-vitro drug release studies of CP NLCs were performed employing a systematic dialysis bag method. The dialysis membrane was activated through a 24 h soaking in purified water (hydration). Following this, the CP NLCs and CP Suspensions formulations were loaded into cellulose membrane dialysis bags with a molecular cut-off of 12-14 kDa (Sigma-Aldrich Co., India). Subsequently, the loaded bags were submerged in 100 mL of pH 4.5 acetate buffer containing a 2% sodium lauryl sulfate solution and subjected to magnetic stirring at 100 rpm at 37 °C. Samples were extracted from the vessel at specified time intervals, and equivalent volumes of fresh solvent were introduced. The concentrations of CUR and PIP were assessed spectrometrically using a UV-visible spectrophotometer (Shimadzu 1800, Shimadzu Japan) at wavelengths of 429 nm (for CUR) and 345 nm (for PIP) employing the simultaneous equation method [21].

Ex-vivo intestinal permeation study

An *ex-vivo*intestinal permeability study involving chicken intestinal (jejunum) segments was conducted to assess CP Suspension and CP NLC [22,23]. The small intestine of the chicken was obtained from a slaughterhouse and thoroughly washed with distilled water to eliminate mucous and luminal content. The complete small intestinal segment, spanning from the upper duodenum to the lower ileum, was precisely located and excised. Manual stripping was used to separate the mesentery, and the intestine was thoroughly washed with normal saline (0.9% w/v Sodium chloride). Various segments of the small intestine were delineated, and the chicken intestine was preserved in Tyrode solution. Approximately 10 cm long segments of the jejunum (non-everted tissue) were prepared and filled with a single-unit dose of CP Suspension and CP NLC. The tissue was suspended in 100 ml of phosphate buffer with a pH of 7.4, representing the blood's pH and simulating the lymphatic fluid's pH. The system was agitated at 100 rpm and maintained at 37 °C, with proper aeration provided by O2/ CO2 (95%/ 5%). The study extended over 3 h. At predefined intervals, aliquots were withdrawn, and the pH 7.4 buffer was replenished. The collected aliquots were filtered through a Whatman filter, subsequently diluted with methanol, and analyzed utilizing a UV-visible spectrophotometer (Shimadzu 1800, Shimadzu Japan) at wavelengths of 429 nm (for CUR) and 345 nm (for PIP) employing the simultaneous equation method [21].

Lymphatic uptake study

The lymphatic uptake of NLCs was assessed in a systematic manner. The cleaned tissue segment was first exposed to a 20 µg/mL solution of the lymphatic uptake blocker PF68, ensuring proper aeration. After a systematic incubation period of 1 h, the NLC formulation was introduced to the tissue using the same systematic procedure as previously described. The permeability of the formulations was then compared between untreated tissue and PF68-treated tissue, providing a clear evaluation of the lymphatic uptake of CP NLCs [20,23].

Results and Discussion

In-vitro drug release study

The saturation solubility and solution stability studies were performed as a part of the pre-formulation study. The study revealed that CUR has maximum solubility in phosphate buffer pH 6.8, whereas PIP has maximum solubility in pH 4.5 acetate buffer. CUR and PIP have negligible solubility in alkaline pH buffers (pH 7.4 and 7.5). The pH-dependent stability studies revealed that CUR is unstable at alkaline pH, whereas PIP is stable at all pH levels. Hence, pH 4.5 acetate buffer was evaluated for *in-vitro* drug release studies. The release profiles of CUR from the Suspension and CP NLCs were 57.91 ± 1.24% and 91.55 ± 0.85%, respectively.

Similarly, the release of PIP from the Suspension and CP NLCs was 100.21 ± 0.19% and 98.11 ± 0.78% after 24 h (Figure 1). Due to the poor solubility of CUR, complete release was not achieved, whereas complete release of PIP was observed before 16 h owing to its high solubility. The initial rapid release of both CUR and PIP can be attributed to the presence of actives in liquid lipids, followed by a slower release attributed to the presence of a solid lipid core. The release profile of CP NLCs exhibited a controlled release pattern compared to CP suspension.

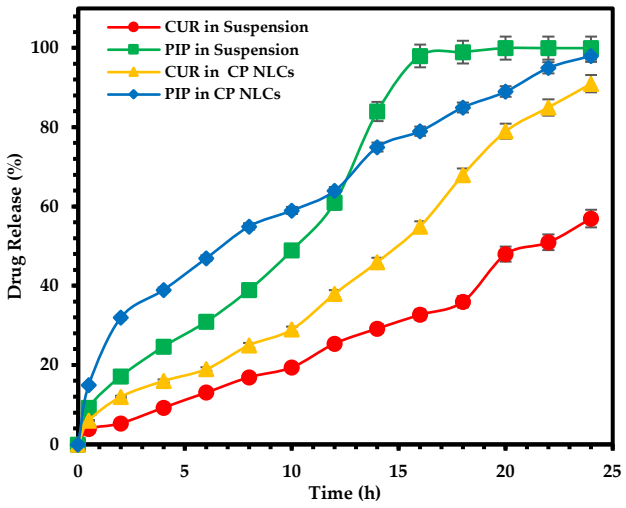

Figure 1. The in-vitro drug release profile of CUR and PIP from both the Suspension and NLC system [Mean ± SD (n=3)].

Ex-vivo intestinal permeation study

The findings of the *ex-vivo* intestinal permeability study, as depicted in Figure 2, are of significant importance. After 3 h of the permeation study, it was observed that the diffusion of CUR and PIP from NLCs was 37.88 ± 2.38% and 37.34 ± 1.54%, respectively. In the case of Suspension, both CUR and PIP diffusion were found to be 26.48 ± 1.97% and 27.08 ± 1.12%, respectively, which is comparatively lower than NLCs. These findings indicate a higher permeation/diffusion occurred with NLCs than with simple Suspension, a crucial discovery in our understanding of drug delivery systems. The enhanced permeation of CUR and PIP from NLCs can be attributed to the presence of nano-sized particles (248.5 ± 12.8 nm) in the formulation and improved permeation facilitated by the surfactant, which reduces the interfacial tension of the formulation [24-26]. The improved permeability may stem from the dissolved state of drugs in lipids and the presence of nano-sized lipid carriers, which increases the surface area. This combination results in a higher drug dissolution and diffusion rate, consequently enhancing permeability [27].

Moreover, it's important to note that NLCs play a crucial role in drug delivery. They can traverse the mucous layer and release the drug directly on the surface of the cell membrane [28]. Existing literature proposes several mechanisms for the enhanced permeability of lipid-based systems following oral administration. These mechanisms include increased membrane fluidity, the opening of tight cellular junctions, inhibition of P-glycoprotein and CYP450 by surfactants, and the stimulation of lipoprotein/chylomicron production by lipids. The latter is particularly significant as lipoproteins/chylomicrons are primarily absorbed by M cells in Peyer's patch [29-31]. The enhanced permeability of CP NLCs may be attributed to the inhibitory action of G50/13 on the P-glycoprotein efflux pump [32,33], resulting in increased absorption after oral administration.

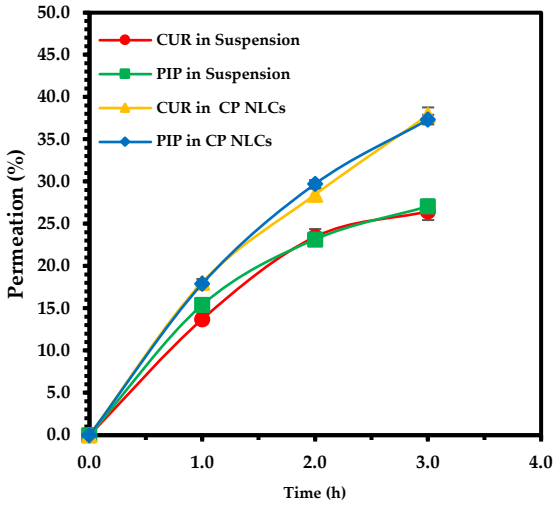

Figure 2. The ex-vivo intestinal permeability of CUR and PIP from both the Suspension and NLC system [Mean ± SD (n=3)].

Lymphatic uptake study

Based on the results of the ex-vivo intestinal permeability study, it was found that the presence of the lymphatic uptake blocker PF68 led to 9.29 ± 1.78% and 5.69 ± 0.59% drug permeation for CUR and PIP, respectively. However, in the absence of PF68, these values increased to 37.88 ± 2.38% and 37.34 ± 1.54% (Figure 3). This indicates a 4.07-fold and 6.56-fold increase in drug permeation for CUR and PIP, respectively, suggesting significant lymphatic transport. The presence of lipids in the NLC formulation primarily contributed to this enhanced lymphatic transport [32,33]. A similar study has addressed the lymphatic uptake of solid lipid nanoparticles in both the presence and absence of P188 [34].

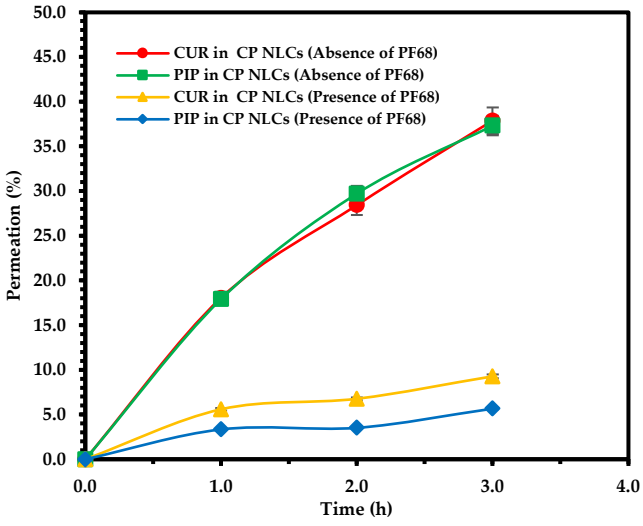

Figure 3. Ex-vivo lymphatic uptake of CUR and PIP from NLC system in presence and absence of lymphatic uptake blocker PF68 [Mean ± SD (n=3)].

Conclusion

The ex-vivo study unequivocally established the critical role of the lymphatic route in significantly enhancing the oral bioavailability of CUR and PIP in the presence and absence of a lymphatic uptake inhibitor. Consequently, CP NLCs unquestionably stand out as a promising drug delivery vehicle for augmenting the oral bioavailability of CUR and PIP. This undoubtedly holds the potential to enhance patient compliance, decrease the required dosage, and ultimately lower the overall cost of therapy.

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Authors contribution

All the authors have contributed equally.

Declaration of interest

The authors declare no conflict of interest.

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