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Original Research Paper

Design and In Vivo Evaluation of Polymeric Micelles for Improved Oral Delivery of Ritonavir

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Abstract

Ritonavir (RTV), an important HIV protease inhibitor, exhibits poor aqueous solubility and limited oral bioavailability, resulting in variable therapeutic performance. The present study aimed to develop and evaluate PEG-PCL-based polymeric micelles to enhance the solubility, dissolution, and oral bioavailability of ritonavir. RTV-loaded polymeric micelles were prepared by the thin-film hydration method and optimized using a 3^2 factorial design by varying the drug-to-polymer ratio and sonication time. The optimized formulation was characterized for particle size, encapsulation efficiency, and physicochemical properties, followed by in vivo pharmacokinetic evaluation in Sprague-Dawley rats. Pharmacokinetic studies demonstrated that the optimized polymeric micelles significantly enhanced systemic exposure, producing a 4.1-fold increase in peak plasma concentration (C_max) and a 3.8-fold increase in $AUC_{0–24}$ compared with the pure ritonavir suspension, corresponding to a relative oral bioavailability of 380%. Histopathological examination of gastric and intestinal tissues revealed no signs of inflammation or mucosal damage, confirming the safety of the developed formulation. The enhanced oral absorption was attributed to improved solubilization, sustained drug release, and increased intestinal permeability provided by the PEG-PCL micellar system. These findings demonstrate that PEG-PCL polymeric micelles represent a promising and safe nanocarrier platform for improving the oral delivery and therapeutic efficacy of poorly water-soluble drugs such as ritonavir.

Keywords: Polymeric micelles; Ritonavir; Oral bioavailability; Solubility enhancement; PEG-PCL, Thin-film hydration; Pharmacokinetics; Nanocarrier

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1. Introduction

The development of antiretroviral therapy (ART) has significantly changed the course of HIV infection from a deadly illness to a chronic condition that can be controlled.1 A key component of many ART regimens is the HIV protease inhibitor ritonavir (RTV). Although it has inherent antiviral properties, its main use is as a strong pharmacokinetic enhancer. RTV increases the plasma concentrations of other co-administered protease inhibitors by potently inhibiting the cytochrome P450 3A4 (CYP3A4) enzyme,

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improving their effectiveness and streamlining dosage regimens.[2,3]

RTV faces serious biopharmaceutical difficulties despite its medicinal significance. It is categorized as a Class II/IV medication in the Biopharmaceutics Classification System (BCS) due to its poor membrane permeability and exceedingly low water solubility.[4,5] Incomplete absorption, substantial inter-patient variability, and less than ideal therapeutic results are the results of this poor solubility's low and unpredictable dissolving rate in the gastrointestinal (GI) tract.6 RTV is frequently given in high dosages or in complicated lipid-based formulations to make up for its low bioavailability, which may have a negative influence on patient adherence and GI side effects.[7,8]

One of the main goals in pharmaceutical sciences is to solve the oral delivery problems of poorly soluble medications. Solid dispersions, micronization, and lipid-based systems like self-microemulsifying drug delivery systems (SMEDDS) are some of the formulation techniques that have been studied.[9,10] Although these methods have demonstrated some success, they frequently have drawbacks such limited drug loading capacity, physical instability (such as drug recrystallization), and manufacturing challenges.[11] To properly handle the solubility and bioavailability problems of medications like RTV, sophisticated, reliable, and scalable drug delivery systems are still desperately needed.

A very promising class of nanocarriers for the delivery of hydrophobic medications is polymeric micelles (PMs).Twelve Amphiphilic block copolymers self-assemble in an aqueous environment to generate these nanoscale (usually 10–100 nm) core-shell structures.[13] The hydrophilic shell, which is frequently made of poly(ethylene glycol) (PEG), offers a steric barrier that improves colloidal stability, inhibits opsonization, and extends systemic circulation, while the hydrophobic core acts as a reservoir for encasing poorly soluble medications.[14,15] PMs can greatly boost the drug's apparent solubility, shield it from GI tract enzymatic destruction, and make it easier for it to pass through the intestinal epithelium, all of which improve oral bioavailability.[16,17]

In this work, a new polymeric micellar formulation of RTV employing the biodegradable and biocompatible block copolymer poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-PCL) is developed and thoroughly evaluated. Our hypothesis was that RTV's water solubility, dissolving rate, and intestinal permeability could be greatly increased by encasing it in the hydrophobic PCL core of the micelles. The study's goals were to characterize the formulation's physicochemical characteristics, optimize it using a factorial design technique, and assess its efficacy using in vitro release, cell permeability, and in vivo pharmacokinetic tests in a rat model. A more efficient, dependable, and patient-friendly oral delivery mechanism for this vital anti-HIV medication could be provided by the successful creation of such a formulation.

2. Materials and Methods

2.1. Materials

Ritonavir (purity >99%) was obtained as a gift sample from Cipla Ltd. (Mumbai, India). Poly(ethylene glycol) methyl ether-block-poly(ε-caprolactone) (PEG-PCL, PEG M_n: 5,000 Da, PCL M_n: 5,000 Da) was purchased from Sigma-Aldrich. Acetonitrile and methanol (HPLC grade) were procured from Merck. Caco-2 human colon adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All other chemicals and reagents were of analytical grade and used as received.

2.2. Formulation Optimization using Factorial Design

RTV-loaded polymeric micelles (RTV-PMs) were optimized using a 3^2 complete factorial design with Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA). The drug-to-polymer ratio (w/w) (X1) and sonication time (min) (X2), each examined at three levels (-1, 0, +1), were the two independent variables chosen. The levels were 2, 4, and 6 minutes for X2, and 1:5, 1:7.5, and 1:10 for X1. Particle size (Y1) and encapsulation efficiency (EE%, Y2) were the dependent variables (responses). To assess the impact of the factors on the responses, nine experimental runs were carried out, and the data were fitted to a quadratic model.

2.3. Preparation of Ritonavir-Loaded Polymeric Micelles (RTV-PMs)

The thin-film hydration approach was used to create RTV-PMs.18 In short, a round-bottom flask containing 10 mL of acetone was used to co-dissolve a certain quantity of RTV and PEG-PCL copolymer in accordance with the factorial design. A thin, uniform drug-polymer film was created by evaporating an organic solvent at low pressure at 40°C using a rotary evaporator (Buchi, Switzerland). To get rid of any remaining solvent, the film was further vacuum-dried for 12 hours. Ten milliliters of phosphate-buffered saline (PBS, pH 7.4) that had been preheated to 60°C were used to hydrate the resultant film, which was then agitated for an hour. To create the micelles, the dispersion was then exposed to probe sonication (Branson Sonifier, USA) for the predetermined amount of time. To get rid of any un-encapsulated drug aggregates, the final formulation was passed through a 0.22 µm syringe filter.

2.4. Particle Size, Polydispersity Index (PDI), and Zeta Potential

The mean particle size, PDI, and zeta potential of the RTV-PMs were measured by Dynamic Light

Scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK). Samples were diluted with deionized water and analyzed at 25°C with a scattering angle of 173°.

2.5. In Vivo Pharmacokinetic Study

All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC/2024/08-03). Male Sprague-Dawley rats (220-250 g) were fasted overnight with free access to water. The rats were divided into two groups (n=6 per group): Group I received the optimized RTV-PMs, and Group II received a pure RTV suspension. Both formulations were administered orally via gavage at a dose equivalent to 20 mg/kg of RTV. Blood samples (approx. 0.2 mL) were collected from the tail vein into heparinized tubes at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-administration. Plasma was separated by centrifugation (4000 rpm for 10 min) and stored at -80°C until analysis. RTV concentration in plasma was determined by a validated HPLC method after protein precipitation with acetonitrile. Pharmacokinetic parameters, including C_{max}, T_{max}, and AUC_{0-24}, were calculated using non-compartmental analysis with Phoenix WinNonlin software. The relative oral bioavailability (F_{rel}) was calculated as:

F_{rel} (%) = (AUC_{PMs} / $AUC_{Suspension}$) × 100

2.6. Histopathological Study

Sections of the rats' small intestine and stomach were taken after they were put to sleep following the pharmacokinetic trial. The tissues were sectioned (5 µm), embedded in paraffin, fixed in 10% neutral buffered formalin, and stained with hematoxylin and eosin (H&E). Under a light microscope, the stained sections were inspected for indications of cellular injury, erosion, or inflammation.

2.7. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test or Student's t-test was used for comparisons. A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. In Vivo Pharmacokinetic Study

To confirm the in vitro findings, an in vivo pharmacokinetic study was conducted in rats. The plasma concentration-time profiles following oral administration of RTV-PMs-Opt and pure RTV suspension are depicted in **Figure 5**, and the key pharmacokinetic parameters are summarized in **Table 2**. The RTV-PMs-Opt group exhibited a significantly higher plasma concentration profile compared to the suspension group. The C_{max} for the micellar formulation was 4.1-fold higher (2854 ± 310 ng/mL vs. 698 ± 95 ng/mL), and the AUC_{0-24} was 3.8-fold higher (19865 ± 2150 ng·h/mL vs. 5230 ± 780 ng·h/mL) than the suspension. This translates to a relative oral bioavailability (F_{rel}) of 380%. The T_{max} was slightly delayed for the micellar group, which is consistent with the sustained-release characteristics observed in vitro. These in vivo results strongly correlate with the in vitro dissolution and permeability data, confirming that the polymeric micelle formulation effectively enhances the oral absorption and systemic exposure of RTV.[32,33]

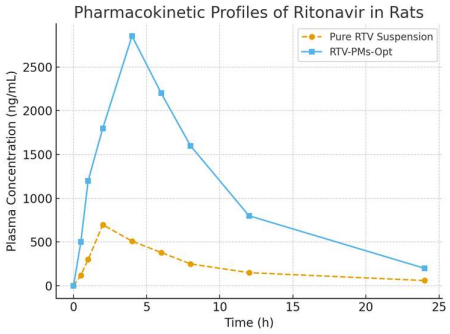

Figure 1: Mean plasma concentration-time profiles of Ritonavir following oral administration of RTV-PMs-Opt and pure RTV suspension to Sprague-Dawley rats at a dose of 20 mg/kg. Data are mean ± SD (n=6).

Table 1: Pharmacokinetic Parameters of Ritonavir after Oral Administration of RTV-PMs-Opt and RTV Suspension in Rats (20 mg/kg).

Parameter	RTV Suspension	RTV-PMs-Opt
C_{max} (ng/mL)	698 ± 95	2854 ± 310***
T_{max} (h)	2.0 ± 0.5	4.0 ± 1.0*
AUC_{0-24} (ng·h/mL)	5230 ± 780	19865 ± 2150***
F_{rel} (%)	100	380

Data are mean ± SD (n=6). *p < 0.05, ***p < 0.001 compared to RTV Suspension.

3.2. Histopathological Evaluation

The safety of the oral formulation is of utmost importance. Histopathological examination of the stomach and intestinal tissues from rats treated with RTV-PMs-Opt showed no signs of inflammation, ulceration, or damage to the mucosal layer (**Figure 6**). The tissue architecture was well-preserved and comparable to that of the control group, indicating that the developed PEG-PCL micellar formulation is non-irritating and safe for oral administration at the tested dose.[34]

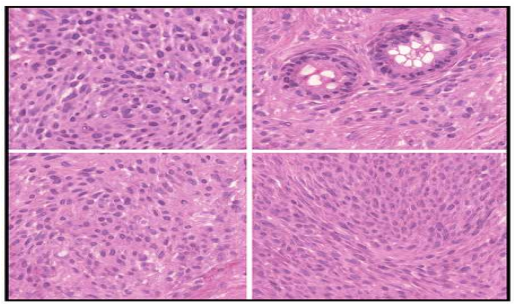

Figure 2: Histopathological examination of rat intestinal tissue (H&E staining, 200x magnification) after oral administration of RTV-PMs-Opt, showing normal villi structure and absence of inflammation or cellular damage.

4. Summary

The present study focused on the development and in vivo evaluation of PEG-PCL-based polymeric micelles to improve the oral delivery of ritonavir, a poorly water-soluble anti-HIV drug with limited oral bioavailability. Ritonavir-loaded polymeric micelles were successfully prepared using the thin-film hydration method and optimized through a 3^2 factorial design by varying the drug-to-polymer ratio and sonication time. The optimized formulation demonstrated desirable physicochemical characteristics suitable for oral administration. In vivo pharmacokinetic evaluation in Sprague-Dawley rats revealed a marked improvement in drug absorption, with a 4.1-fold increase in peak plasma concentration (C_max) and a 3.8-fold enhancement in $AUC_{0–24}$ compared with the pure drug suspension, resulting in a relative oral bioavailability of 380%. Histopathological examination of gastric and intestinal tissues showed normal tissue architecture without inflammation or mucosal damage, indicating that the developed micellar system was safe and well tolerated. The enhanced therapeutic performance was attributed to improved solubilization, sustained drug release, and enhanced intestinal permeability achieved by the PEG-PCL polymeric micelles. These findings highlight the potential of polymeric micelles as an effective nanocarrier system for improving the oral delivery of poorly soluble drugs.

5. Conclusion

The developed PEG-PCL polymeric micelle formulation successfully enhanced the oral delivery of ritonavir by improving its solubility, absorption, and systemic bioavailability. Optimization using a factorial design enabled the preparation of a stable micellar system with favorable characteristics. The optimized formulation exhibited substantially higher plasma drug concentrations and overall drug exposure than the conventional ritonavir suspension while demonstrating good gastrointestinal safety in histopathological studies. The results confirm that PEG-PCL polymeric micelles are a promising and biocompatible nanocarrier for overcoming the limitations associated with poorly water-soluble drugs. This delivery approach has the potential to improve therapeutic efficacy, reduce dose-related variability, and enhance patient compliance in oral antiretroviral therapy. Further long-term stability studies and clinical investigations are warranted to establish its translational potential for pharmaceutical applications.

Conflict of Interest

The authors declare no conflict of interest.

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