Modified Rifampin Nanoparticles: Increased Solubility with Slow Release Rate

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Abstract

Background: Recent advances in nanotechnology-based drug delivery system have been shown to improve either antibacterial efficacy or pharmacokinetics behavior. The aim of this study was to design a rifampin nanoparticle (RIF-NP) which has a high loading capacity with the slow release profile. Material and Methods: The designed chitosan/gelatin/lecithin (Chg/L) RIF-NPs were prepared by multilamellar vesicle. Thereafter, the particle size, zeta potential, morphology, and release rate were investigated. To optimize the loading capacity and release profiles, different concentrations of lecithin were used. Results: Our results showed a correlation of lecithin concentration with size, zeta potential, and loading capacity of RIF-NPs. Increases in lecithin concentration (0.2–2.0 g) could cause a significant size reduction in NPs (250–150 nm); the amount of zeta potential (from 14 to 49 mV; $P < 0.05$) and loading capacity increases from 8% to 20% ($P < 0.05$). Designed NPs had slow drug release profile which was influenced by pH and lecithin concentration. The cumulative percentage of RIF released at pH 7.4 was approximately 93% up to 12 h. In overall, release profile was better than standard drug, even in various pH conditions (pH = 1, 3.4, and 7.4). The Chg/L-RIF NPs may be considered as a promising drug nanocarrier. Conclusions: These NPs release RIF in slow and constant rate, which effectively might eliminate the bacilli and prevent the formation of RIF-resistant bacilli.

Keywords: Chitosan, lecithin, nanoparticle, rifampin, slow release

INTRODUCTION

Rifampin (RIF), a semisynthetic derivative of rifamycin, is a macrocyclic antibiotic with broad antibacterial activity which is produced from Amycolatopsis rifamycinica.[1] Its usages have been approved in the therapy of tuberculosis together with isoniazid, ethambutol, and pyrazinamide.[2,3] Under normal conditions, RIF is quickly absorbed from the gastrointestinal tract.[4] Usually, RIF dose is 600 mg which taken on fasting and will reach the maximum in serum concentration (8–24 mcg/ml) within 2 h of administration.[5,6] In contrast, the absorption of this drug in intestinal tract takes longer time (up to 5 h), or the absorption may sometimes become incomplete if they are taken by food or antacids.[7] Practically, RIF is the main anti-tuberculosis drugs, although its low solubility and absorption necessitates an alternate drug delivery/carrier system.[4,10,11] Recently, with advance in nanosuspension technology, many investigators look for selective RIF delivery mechanisms that target Mycobacterium tuberculosis inside the macrophages.[4,12-14] In all these studies, the main focus of the investigation was increasing the RIF solubility in aqueous solution. For this purpose, they used chitosan (CS) and polyethylene glycol (PEG) as a surface coating.[4,9,14] In overall, most of illustrated drug delivery systems were successful in either increasing the antituberculous efficacy of RIF or improving its pharmacokinetics.[14,16] Kalluru et al.[16] designed an orally encapsulate RIF-nanoparticles (NPs) and used it against infected mice. They showed significant pharmacokinetics improvement of RIF in comparison to conventional doses.[14] In another study, RIF was loaded on solid lipid NPs which composed of cetyl palmitate,

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Materials and Methods

Reagents

RIF was from Sigma–Aldrich (St Louis, MO, USA); acetonitrile and phosphoric acid were high-performance liquid chromatography (HPLC) grade (BDH Chemicals, Poole, UK). CS and gelatin were purchased from Merck by high purity. Deionized water produced by the Milli-Q system (Bedford, MA, USA) was utilized. All other chemicals and reagents were analytical grade.

The analysis of rifampin by high-performance liquid chromatography

The analysis of RIF was done using the Knauer (Germany) HPLC system equipped with K-1001 pump, K-2008 PDA detector, a manual injection valve (Rheodyne, USA) with a 20 µL loop, degasser, and C18 Eurospher column (250 mm × 4.6 mm, 5 µm, 100 Å). The RIF was eluted under isocratic mode using two different solutions, including solvent A - acetonitrile (50%, v/v), solvent B - buffer phosphate (pH = 3.2) (50%, v/v) at room temperature. The flow rate of the mobile phase was 1 ml/min, and detector monitored the samples at 335 nm.

Preparation of rifampin-lecithin loaded on chitosan-gelatin nanoparticles

Chg/L RIF-NPs were prepared through multilamellar vesicle method. Briefly, 2 g lecithin was dissolved in 10 ml of chloroform, and 300 mg of RIF was added in this solution. The obtained solution was dried by rotary evaporation to obtain the dried thin film. The solution comprises 3% CS solution and 1% gelatin solution (4:1 v/v) which was added dropwise and stirred continuously for 3 h using glass balls. To produce NPs, 10 ml of 1% tripolyphosphate (TPP) solution was added dropwise, and the reaction mixture was allowed to stir for 2 h. Then, the NPs solution was centrifuged under 10,000 rpm for 10 min and the sediment was stored at −80°C for future analysis.

Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy with Elmer infrared spectrometer (model FT-PC-160) at a range of 4000–400 cm⁻¹ was used to analyze the samples. Briefly, a small amount of samples were mixed with KBr and compressed to form tablets. Then, the tablets were scanned in transmission model, using a resolution of 4 cm⁻¹.

Loading capacity

To determine the loading capacity of RIF, it is necessary to destroy the structure of NPs for RIF’s release. For this reason, 2 ml of acetonitrile was added into 2 mg of lyophilized NPs and the RIF release rate was determined using the calibration curve. Then, the percentage of loading capacity was calculated according to the following equation:

\[
\text{Loading capacity (\%)} = \left( \frac{\text{[RIF encapsulated/NPs total]}}{100} \right)
\]

Size measurement and determination of zeta potential

The dynamic light scattering using Zetasizer (Nano-ZS; Malvern Instruments, Malvern, UK) was used to determine the size and zeta potential of the Chg/L RIF-NPs. In addition, the topography of the NPs was investigated using the transmission electron microscopy, (JEM1010-JEOL).

In vitro release studies

The drug release was studied according to our previous work. Briefly, an equal amount of Chg/L RIF-NPs and conventional RIF was put into two separate dialysis bags (cut-off 14 KD) that were immersed in different pH buffer solutions (pH 1.00, 3.20, and 7.40) under continuous stirring at 37°C. To keep the volume constant, dissolution medium was replaced with exact amount of samples that were withdrawn at predefined time. The HPLC method was used to get the final RIFs’ release from samples.

Statistical analysis

The experiment was done using three replications for each sample. For statistical analyses, the Statistical Package for the Social Sciences software (SPSS version 22, Armonk, NY) was used and each result presented in this study was a mean ± standard deviation (vertical bars) of three replications.

Results

Physicochemical characterization of chitosan/gelatin/lecithin rifampin-nanoparticles

The particle size, zeta potential, and loading capacity of Chg/L RIF-NPs are shown in Table 1. The results indicated that with increases in lecithin concentration, the particle size values decreased from 50–250 to 50–150 nm. Moreover, lecithin concentration influenced on loading capacity of RIF. Furthermore, with increasing lecithin concentration, the amount of the zeta potential was increased from 14 to 49 mV.

In all experimental conditions, NPs have the multibranch morphology [Figure 1].

Fourier transform infrared analysis

Figure 2 shows the FT-IR spectra of (a) CS, (b) gelatin, (c) RIF, and (d) Chg/L RIF-NPs. The FT-IR spectrum of RIF showed several characteristic peaks: absorption band of O-H and N-H at 3452 cm⁻¹, N–CH₃ band at around 2942 cm⁻¹, absorption band at about 1697 cm⁻¹ for acetyl –C=O, –C=N asymmetric stretching at 1647 cm⁻¹, and C=C stretching at 1564 cm⁻¹. The peaks unique to CS were observed at 3439 cm⁻¹ (OH), 2916 cm⁻¹ (CH), 1636 cm⁻¹ (NH group), and 1639 cm⁻¹ (C=O

Tween 80, and Poloxamer 188. This complex was 8 times more effective than RIF’s solution against Mycobacterium fortuitum. In our previous investigation, we showed a combination of phosphoglycerides, CS, and gelatin NPs (CS protein-lipid NPs), as a new carrier for gene transfer and drug delivery system. Herein, we tried to formulate and optimized chitosan/gelatin/lecithin (Chg/L) RIF-NPs. Lecithin is a safe and biocompatible phospholipids mixture which has been reported to be useful in various delivery nanosystems. The designed NPs with high loading capacity and slow release profile would be an ideal carrier for RIF delivery system.
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Table 1: Physicochemical properties of NPs

| Particle type | Z-average (nm) \( n=3, \text{Mean±SD} \) | Zeta potential (mV) | RIF loading (\% (w/w)) | Encapsulation efficiency (\%) |
|---------------|------------------------------------------|----------------------|-------------------------|-----------------------------|
| A             | 50-250                                   | 14                   | 8                       | 31                          |
| B             | 50-150                                   | 49                   | 20                      | 67                          |

A: The amount of Lecithin was 0.2g at 10 mL chloroform. The ration of Chitosan to Gelatin is 3:1 (v/v %) B: The amount of Lecithin was 2g at 10 mL chloroform. The ration of Chitosan to Gelatin is 3:1 (v/v %)

In vitro release studies

The release of RIF form NPs in various lecithin concentrations (0.2–2.0 g at pH = 7.4) and different pH levels (i.e., 1.0, 3.4, and 7.4) was determined. All the release behavior lasted for 24 h. Replicated experiments (thrice for each test) were performed for all tests and the mean of results was shown as a function of time as shown in Figures 3 and 4 and Tables 2-4. The results showed that at higher concentration of lecithin (2.0 g), the RIF release was 10-fold increased [Figure 3]. In addition, at pH 7.2, the cumulative percentage of RIF released was notably higher compared to pH 3.4 and 1.0 and reached approximately 93% up to 12 h [Figure 4a]. Furthermore, the comparison of Chg/L RIF-NPs with conventional RIF indicated lesser release rate (more than 3-fold) in standard RIF than Chg/L RIF-NPs [Figure 4b].
**Table 2: The release of rifampin (µg/mL) at different time (pH=1.0)**

| Particle type | 15 min | 30 min | 1 hr  | 2hr  | 4 hr  | 6 hr  | 9 hr  | 12 hr | 24 hr |
|---------------|--------|--------|-------|------|-------|-------|-------|-------|-------|
| Rifampin NPs  | 0.80   | 1.53   | 3.37  | 4.08 | 4.32  | 4.42  | 4.53  | 4.66  | 4.82  |
| Standard drug | 0.11   | 0.13   | 0.19  | 0.38 | 0.88  | 1.28  | 1.51  | 2.3   | 2.38  |

**Table 3: The release of rifampin (µg/mL) at different time (pH=3.4)**

| Particle type | 15 min | 30 min | 1 hr  | 2hr  | 4 hr  | 6 hr  | 9 hr  | 12 hr | 24 hr |
|---------------|--------|--------|-------|------|-------|-------|-------|-------|-------|
| Rifampin NPs  | 0.44   | 0.53   | 0.72  | 1.25 | 3.10  | 3.63  | 5.48  | 6.61  | 6.61  |
| Standard drug | 0.41   | 0.57   | 0.70  | 0.75 | 0.75  | 0.75  | 0.75  | 0.77  | 0.75  |

**Table 4: The release of rifampin (µg/mL) at different time (pH=7.2)**

| Particle type | 15 min | 30 min | 1 hr  | 2hr  | 4 hr  | 6 hr  | 9 hr  | 12 hr | 24 hr |
|---------------|--------|--------|-------|------|-------|-------|-------|-------|-------|
| Rifampin NPs  | 0.80   | 2.92   | 5.84  | 16.80| 27.41 | 31.41 | 34.45 | 36.96 | 36.98 |
| Standard drug | 0.97   | 2.01   | 2.39  | 2.10 | 8.61  | 8.66  | 12.36 | 12.62 | 13.11 |

**Discussion**

Because RIF is poorly water soluble and has low adsorption,[31] it is necessary to take a high doses in order to reach a therapeutic plasma concentration.[12] This poor solubility in aqueous media was improved using hydrophilic compounds such as CS, PEG, and lecithin as a surface coating.[32-35] Herein, RIF solubility was increased with increasing lecithin concentration. Lecithin is a mixture of phosphatides, and since it is safe and biocompatible, it could be used as solubilizing carrier.[20,21] We showed two important roles for lecithin in our experimental setup: it increases RIF solubility and it helps to synthesize the NPs by reducing the use of TPP in formation of NPs. TPP frequently used in the formulation of NPs, although the surface charges of particles depend on volume and concentration ratio of complex and TPP.[35] To reach the optimal concentration, we added TPP in dropwise manner to complex solution under steady stirring.[18,35] In addition to lecithin, we had used combination of CS and gelatin to make RIF-NPs. This complex could improve both absorption and bioavailability of the RIF. The CS has positive charges because of the hydroxyl and NH\(^+\)\(_3\) groups, and gelatin has negative charges because of the hydroxyl and COO\(^-\) groups. The electrostatic and hydrogen bonding interactions[36-38] cause their attachment, together. Thereby, the synthesized NPs were based on electrostatic interactions between the negatively charged of lecithin exciting to a phosphate group and positively charged amino groups (NH\(^+\)\(_3\)) of CS-gelatin.[19]

In general, the size of particles is considered as an important property that will affect in vivo performance of NPs. In the present study, the concentration of lecithin was optimized to obtain small NPs with high zeta potential and high loading capacity. As shown in Table 1, different concentrations of the lecithin (0.2 and 2 g at 10 ml chloroform) were used to optimize the size of particles. The results showed that with increases in lecithin concentration, the particle size values decreased. Because the NPs formed by electrostatic interaction of the negative charge of lecithin with the positive charge of CS-gelatin. As the amount of lecithin increases, the electrostatic interaction increases, causing the reduction in NPs sizes. Thereby, with 0.2 g lecithin, the size of particles was between 50 and 250 nm, and with 2 g lecithin, the size reduces to 50–150 nm. In addition, with increasing lecithin concentration, the amount of the zeta potential was obviously increased. As the value of zeta potential was higher than +30 mV, the stability of NPs suspensions was assured.[18] The positive values obtained for zeta potential indicated that the NPs surface was positively charged. This may be due to the availability of CS free NH\(^+\)\(_3\) groups on the complex.[19] The increase in the concentration of lecithin affected the drug solubility, i.e., higher lecithin causes higher RIF solubility. In all experimental conditions, NPs have the multibranch morphology.

The release rate is quite slow and gets 12 and 16% after 12 h, respectively [Figure 4a]. The rate of RIF released at pH 7.2 is notably higher compared to pH 3.4 and 1.0. The cumulative percentage of RIF released at pH 7.2 was approximately 93% up to 12 h [Figure 4a]. Furthermore, the comparison of RIF-lecithin loaded on NPs with conventional RIF indicated lesser release rate (more than 3-fold) in standard RIF than RIF-lecithin loaded on NPs [Figure 4b].

The release of RIF from Chg/L RIF-NPs showed a slow and constant release rate, which depends on pH and lecithin concentration. In higher concentration of lecithin, the RIF release was 10-fold increased [Figure 3]. The release of RIF from NPs (2.0 g lecithin) was started after 15 min which was about 24.0 µg/ml; after 2 and 6 h, the release of RIF was about 295.5 µg/ml and 890.4 µg/ml, respectively; and at 12 h, the release reached to 1109.5 µg/ml. Hence, these results indicate that with increase lecithin concentration, the RIF release is increased. At pH 1.0 and 3.4 (close to stomach pH), the release rate is quite slow and gets 12 and 16%, respectively, when it is approaching to 12 h and thereafter a sustained release was observed up to 24 h [Figure 4a]. The rate of RIF...
released at pH 7.2 is notably higher compared to pH 3.4 and 1.0. The cumulative percentage of RIF released at pH 7.2 was approximately 93% up to 12 h [Figure 4a]. Furthermore, the comparison of Chg/L RIF-NPs with conventional RIF indicated that the release of RIF from Chg/L RIF-NPs was higher than standard drug RIF (more than 3-fold) [Figure 4b].

Conclusions
The method of preparation and formulation of (Chg/L) RIF-NPs was simple, reproducible, and cost-effective. These NPs with high loading capacity and slow release pattern might facilitate the target nano-delivery drug systems, which needs further investigation.

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Conflicts of interest
There are no conflicts of interest.

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