CHARACTERIZATION AND PHARMACOKINETICS OF RIFAMPICIN LOADED POLY-ε-CAPROLACTONE NANOPARTICLES FOR ORAL ADMINISTRATION

1Atanu Kumar Behera*, 2S. Shah, 3Shubhrajit Mantry, 4B.B. Barik, 5Snehal A.Joshi

1,2Department of Pharmacy, Monad University, Hapur, India
3Department of Pharmaceutics, Kottam Institute of Pharmacy, AP, India
4Department of Pharmacy, Jazan University, Saudi Arabia
5Dadhichi College of Pharmacy, Cuttack, Odisha, India

ABSTRACT

The objective of the research work was to develop and characterize rifampicin (RIF) loaded Poly-ε-caprolactone nanoparticle (PCL NP). PCL NP containing RIF were prepared using single emulsion solvent evaporation method. The formulations were characterized through transmission electron microscopy (TEM), size and size distribution analysis, polydispersity index (PDI), zeta potential, percent drug entrapment, percent nanoparticulate yield and in vitro drug release. The formulations were further characterized for pharmacokinetic study and in vivo biodistribution. The nanoparticles were found to be spherical in shape. The size of nanoparticles was found to be 219±12.2nm with low PDI suggesting narrower particle size distribution. In vivo biodistribution study showed higher localization of RIF loaded GPs in various organs, as compared to plain RIF’s solution in PBS (pH 7.4). Contrary to free drug, the nanoparticles not only sustained the plasma level but also enhanced the AUC and mean residence time (MRT) of the drug, suggesting improved pharmacokinetics of the drug. Hence, PCL NPs hold promising potential for concern reducing dosing frequency with the interception of minimal side effects.

Key words: Rifampicin, Nanoparticle, Polydispersity index, Poly-ε-caprolactone, TEM

Corresponding Author:
Atanu Kumar Behera
atanukumarbehera@gmail.com

Available online: www.ijipsr.com  September Issue
INTRODUCTION

Tuberculosis (TB) caused by Mycobacterium tuberculosis (M. tuberculosis), a ubiquitous, highly contagious chronic granulomatous bacterial infection, is still a leading killer of young adults worldwide. TB has returned with a new face and the global scourge of multi-drug resistant TB (MDR TB) is reaching epidemic proportions. The potential of site specific drug delivery in optimizing drug therapy [1] has given impetus to significant advancements in the pharmaceutical engineering of novel dosage forms such as nanoparticles, which are solid colloidal polymeric carriers less than 1 mm in size [2]. Nanotechnology plays an important role in therapies of the future as “nanomedicine” by enabling this situation to happen, thus lowering doses required for efficacy as well as increasing the therapeutic indices and safety profiles of new therapeutics. Rifampicin (RIF) is the first line drug currently used for treatment of latent M. tuberculosis infection in adults. But a number of side effects like lack of appetite, nausea, hepatotoxicity, fever, chill, allergic rashes, itching and immunological disturbances, patient non-compliance with long term therapy limits its use [3,4]. Thus, the current strategy for enhancing the therapeutic activity of currently available drugs is to entrap drugs within a delivery system from where they are slowly released over an extended time period [5].

In the present study, RIF-loaded PCL NPs were prepared by single emulsion solvent evaporation method and the PCL NPs were characterized by various physicochemical means such as size measurements, drug entrapment and in vitro drug release. The optimized formulations were further evaluated for pharmacokinetic parameters and in vivo biodistribution studies were performed on rat model.

MATERIAL AND METHOD:

Material:
Polycaprolactone (Av. M_w 60,000) was purchased from Sigma-Aldrich (Mumbai, INDIA); Rifampicin was a gift sample from Macleod Pharmaceuticals, Mumbai. Polyvinyl Alcohol (Av. M_w 22000), ascorbic acid, was purchased from Qualichens, New Delhi. Dichloromethane (DCM) and Acetonitrile, BHT, n-pentane (analytical grade) were purchased from Merck (India).
Method:
PCL Nanoparticles were prepared using single emulsion solvent evaporation method [6]. Organic phase was prepared by dissolving 300 to 500 mg of the PCL in 10 ml of water immiscible organic solvent (Methylene chloride, DCM). Subsequently, 20 mg of Rifampicin (RIF) was added & dissolved in the solvent completely. The aqueous phase was prepared by dissolving 2.5g of PVA in 100 ml of PBS having pH 7.2, which was previously filtered through 0.22 µm cellulose nitrate membranes. Upon addition of the PCL/RIF solution into 40 ml of PVA solution under ultrasonication using a Vibra-Cell probe sonicator (VC 5040, Sonics and Materials, USA) for a specific time (1, 3, and 5 min), an oil-in-water nano-emulsion is formed. The nano-emulsion is next poured into 40mL aqueous solution of 2.5% (w/v) PVA and the final emulsion was stirred for six hours at 300 RPM at room temperature on a magnetic stir plate to allow the evaporation of DCM and ACN and to allow the formation of the nanoparticles. The resulting nanoparticle suspension is centrifuged twice at 11,000 RPM and two washing cycles to remove the non-encapsulated RIF and DCM trace. Free drug gets settled down. The nanoparticulate suspension was then kept in deep-freezer for suitable time. Lyophilization was then carried out for 24 hours.

Characterization of nanoparticles
Shape
Transmission Electron Microscopic studies (TEM)
NPs were also evaluated for morphology by a transmission electron microscope (Philips/FEI Inc, Barcliff, Manor, NY). For this purpose, a sample of drug loaded NPs (0.5 mg/ml) were suspended in water and sonicated for 30 seconds. One drop of this suspension was placed over a carbon coated copper TEM grid (150 mesh, Ted PELLA Inc., Rodding, CA) and negatively stained with 1 % uranyl acetate for 10 minutes and then allowed to dry. Images were visualized at 120 kV under microscope.

Size and Zeta Potential
To determine the particle size and zeta potential, ~1 mg/ml of drug loaded NP solution was prepared in double distilled water. 100 µl of the sample was diluted to 1 ml, sonicated in an ice bath for 30 seconds and subjected to particle size and zeta potential measurement using the Zetasizer (Zeta Sizernano, Nano ZS, ZEN3600, Malvern Instrument, UK).
**Entrapment Efficiency**

Entrapment efficiency of nanoparticles was determined by the method proposed by Vandervoort and Ludwig [7]. The amount of RIF entrapped was determined by incubating the nanoparticle suspension (1.0 ml) in 5.0ml phosphate buffer saline (PBS, pH 7.4) for 2 h at 500 rpm at 25±1 °C on a magnetic stirrer (Remi, Mumbai, India). The amount of un-entrapped drug was determined spectrophotometrically (Shimadzu, 1601; Kyoto, Japan) in the supernatant obtained after separation of nanoparticles by centrifugation at 10,000×g for 30 min by using Eq. (1):

\[
\text{Drug entrapment (\%)} = \frac{\text{Amount of drug used in formulation} - \text{amount of unbound drug}}{\text{Amount of drug used in formulation}} \times 100
\]

The purified nanoparticulate suspension was ultracentrifuged (Remi, Mumbai, India) at 10,000×g for 1 h at 4±1 °C. The supernatant was discarded and the pellet was freeze dried. The yields of NPs were calculated using Eq. (2):

\[
\text{Nanoparticles yield (w/w\%)} = \frac{\text{Amount of recovered NPs}}{\text{Total amount of polymer and drug added}} \times 100
\]

Drug loading was calculated using Eq. (3)

\[
\text{Drug loading (w/w\%)} = \frac{\text{Amount of drug in NPs}}{\text{Amount of NPs recovered}} \times 100
\]

**In Vitro Release Study**

In vitro release studies from NPs was determined in PBS buffer (0.01 M, pH 7.4), containing 1% Na CMC and 0.02% w/v ascorbic acid was taken as the dissolution medium to prevent the degradation of rifampicin.) at37°C ± 0.5°C for Rifampicin. The NP suspension was equally divided in three tubes containing 5ml each. These tubes were kept in a shaker at 37 °C and 150 RPM (WadegatiLabequip, India). At particular time intervals, these tubes were taken out from shaker and centrifuged at 13,800 RPM, 4 °C for 10 minutes (REMI 1-16K, Mumbai). The supernatants were taken out to estimate the amount of drug release, at that particular time by using a spectrophotometer (UV-1700, Schimadzu). To the residue same amount of fresh PBS (0.01 M, pH 7.4, containing 1% w/v Na CMC and 1% w/v ascorbic acid) was added and kept in shaker for further study [8].
**Determination of Rif in Blood and Tissues**

For a 100µl rat plasma sample (ice cold) 50 µl of ACN (BHT 0.02%) were added. After vortex mixing (30 s) 3ml of dichloromethane–n-pentane (1:1) (BHT 0.02%) were used for extraction of Rif by vortexing for 60 s. The mixture was centrifuged at 2604 × g for 5 min (4°C), the organic layer transferred to a glass conical tube and evaporated to dryness in a vortex evaporator. The extraction procedure was repeated and the total residue obtained was reconstituted in 100 or 200 µl of ACN (BHT 0.02). Aliquots of these solutions were injected into the chromatograph after Millipore Millex-HV filtration (0.45 µm) [9].

**Tissue**

With a 0.5 g of rat tissue (liver and lungs) sample, 2ml of sodium phosphate buffer (pH = 4.5) containing 10⁻³M sodium ascorbate was added. The sample was homogenized by using an ultraturrax-T25 dispersing apparatus (16,000 RPM, 3 min) (IKA® India Private Limited). An aliquot of the homogenate (300 µl) was transferred to a glass conical tube, placed on ice, and 50 µl of ACN (BHT 0.02%) were added and vortex mixed (30 s). The mixture was extracted with 3ml of dichloromethane, n-pentane (1:1) (BHT 0.02%) by vortexing for 60s. After centrifugation at 2604×g for 5 min (4 °C), the supernatant was transferred to a glass conical tube and evaporated to dryness in a vortex evaporator. The extraction procedure was repeated and the total residue was redissolved in 200 µl of ACN (BHT 0.02%). After filtration, an aliquot of this solution (50 µl) was injected into the HPLC system [9].

**Bioavailability and Pharmaceutics Study**

Healthy male albino Wistar rats of 150-250 g body weight were used for pharmacokinetic study. The study was approved by the Institutional Animal Ethical Committee of Sapience Bioanalytical Research Laboratory, Bhopal, India, registered under CPCSEA, (Registration No. 1413/a/15/CPCSEA). Animals were randomly divided into four groups and twelve each. Native drug and formulated nanoparticle were orally administered as a suspension using an oral gavage needle. For the native and nanoparticles of Rifampicin of 12 mg/kg body weight [10] of rat respectively were administered for the evaluation of pharmacokinetic parameters. Group I: PCL NPs containing RIF given orally. Group II: Aqueous RIF solution orally (conventional dose). Group III: Empty nanoparticulate formulation given orally Group IV: Normal control group. Following the drug administration, animals from each group were bled via the retro-orbital plexus at different time points, plasma was separated and analyzed for the drug. In addition, the
animals were sacrificed at various time points and the drug content analyzed in 20% organ homogenates (i.e. 50mg of the organs homogenized in 250μL isotonic saline) of lungs, liver and spleen. The results are expressed as μg drug per ml plasma and percentage of drug distributed to organs after particular time intervals.

**Pharmacokinetic Analysis**

The plasma drug profile was obtained and the data were used to determine various pharmacokinetic parameters. Peak plasma concentration (C\text{max}) and time taken to reach C\text{max}, (T\text{max}) were computed from the curve. The area under the concentration–time curve (AUC\text{0–t}) was determined by the trapezoidal rule. The terminal AUC\text{t–∞} was obtained by dividing the last measurable plasma drug concentration by the elimination rate constant (obtained by regression analysis). The sum of AUC\text{0–t} and AUC\text{t–∞} yielded the total AUC\text{0–∞} while the area under moment curve (AUMC)/area under curve (AUC) gave the mean residence time (MRT).

**RESULT AND DISCUSSION**

**Preparation and Characterization Of Rifl Loaded Pcl Nanoparticles**

PCL Nanoparticles were prepared using single emulsion solvent evaporation method. The average particle sizes of PCL NPs were found to be 219±16.08 nm (Fig. 1). A polydispersity index (PDI) of 0.315±0.04 suggested the narrow size distribution of the nanoparticles. The entrapment efficiency is of 34.95±1.28; yield 61.35±4.62%, practical drug loading 14.85±1.56%, and zeta potential are of -23.11±3.24 (Fig. 2). The TEM photomicrograph revealed that the RIF loaded NPs were spherical in shape (Fig. 3).
In Vitro Drug Release Studies

The in vitro drug release of RIF-loaded PCL NPs was studied for 300 hours at pH 7.4 (Fig. 3). A slow sustained release of Rifampicin due to the entrapment of drugs inside the core of nanoparticles as the surface modification increased [11]. In order to determine the release pattern from the prepared NPs, various in-vitro release kinetic models such as Zero Order, Higuchi and First order were analyzed from first day to the tenth day. In the formulations highest regression co-efficient was observed in zero order equations starting from day one to tenth day. The slower and sustained release of the drug from the beginning can be attributed to the erosion of polymeric matrix which releases the encapsulated drug [12].
In Vivo Drug Distribution Studies

The all the pharmacokinetic parameters were given in table no. 2 after oral administration of both native drug and drug loaded nanoparticles. In vivo pharmacokinetics study also shows a prolong release like the vitro release. The slow and sustained release of RIF from PCL NPs accounts for the long time (Tmax) required for attaining Cmax. The slow elimination rate (Kel) resulted in significantly prolonged t1/2 of RIF compared to native drug. The area under the curve (AUC) corresponds to the integral of the plasma concentration versus an interval of definite time, which was also found to be significantly higher (P≤0.05) as compared to the native drug. Further, AUC values provide an evidence for the increased bioavailability of rifampicin in encapsulated form (NPs) as compared to native RIF. The relative bioavailability of the encapsulated drug was 20 and T_MIC for 196±3.64 hours and the AUC / MIC is 962.145 signifies the capability of the formulation.

Table 2: Pharmacokinetics of RIF for free and RIF encapsulated dosage form at one dose

<table>
<thead>
<tr>
<th>Drug</th>
<th>AUC0-t (hr*µg/ml)</th>
<th>AUC0-Inf (hr*µg/ml)</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (hr)</th>
<th>t1/2 (hr)</th>
<th>Kel (1/hr)</th>
<th>Kel-start (hr)</th>
<th>Kel-end (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin Free Drug</td>
<td>9.018</td>
<td>10.508</td>
<td>1.269</td>
<td>2</td>
<td>2.441</td>
<td>0.055</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>RIF Loaded NP</td>
<td>180.663</td>
<td>192.429</td>
<td>1.439</td>
<td>48</td>
<td>45.561</td>
<td>0.0152</td>
<td>192</td>
<td>264</td>
</tr>
</tbody>
</table>
AUC_{0-t} : Area under Curve from time 0 to time t, AUC_{0-\text{Inf}} : Area under Curve from time 0 to infinity, C_{\text{max}}: Peak plasma concentration, T_{\text{max}} : Time taken to reach C_{\text{max}}, t_{1/2}: half life, K_{el}: Rate constant of elimination, K_{el-start}: Initial K_{el}, K_{el-end}: Final K_{el}.

Figure 5: Plasma drug concentrations of pure RIF after one oral dosing

Figure 6: Plasma drug concentrations of RIF loaded PCL Nanoparticle after one oral dosing

The distribution of drug in different organs clearly indicates the superiority of the nanoparticulate RIF in contrast to the native drug in increasing the accumulation of RIF within the organs rich in macrophages (liver, lungs and spleen). Following a single administration of both the dosage form to rat the concentration of RIF measured after 36 hours for the native drug and 192 hours for the RIF loaded PCL nanoparticle (Fig: 7, 8). Which shows an accumulation of the drug in these organs was achieved significantly higher (P≤0.05) in case of the RIF loaded PCL nanoparticle even after 8 days.
CONCLUSION:
The RIF loaded nanoparticles were successfully prepared by single emulsion solvent evaporation method. The prepared carrier system had particle size in the nanometer range with lower polydispersity index signifying the uniform distribution of the particles. The nanoparticulate system showed a controlled drug release upto 8 days suggesting a possible advantage of a sustained release formulation. The prepared formulation showed an enhanced AUC, MRT and hence bioavailability. Hence, it may be concluded that RIF loaded NPs may be utilized effectively in the management of tuberculosis leading to decrease in frequency of dosing, minimized side effects and improving the therapeutic efficacy of the drug.
ACKNOWLEDGEMENTS

The authors are grateful to Macleod Pharmaceuticals Ltd., Mumbai, India, for providing Rifampicin as a gift sample.

REFERENCE:

