International Journal of Pharmaceutics 532 (2017) 612–622

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Research Paper

Rifampicin Lipid-Polymer hybrid nanoparticles (LIPOMER) for enhanced Peyer's patch uptake

Sagar S. Bachhav[a], Vikas D. Dighe[b], Darsheen Kotak[a], Padma V. Devarajan[a,*]

[a] Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, N.P. Marg, Matunga (E), Mumbai-400019, Maharashtra, India
[b] National Center for Preclinical Reproductive and Genetic Toxicology, ICMR-National Institute for Research in Reproductive Health (NIRRH), J.M. Street, Parel, Mumbai-400 012, India

ARTICLE INFO

Keywords:
M-cell
Rifampin
Bioadhesion
Poly (methyl vinyl ether-co-maleic anhydride)
Design of experiments
Tuberculosis
Lymphatic system

ABSTRACT

The oral uptake of intact nanocarriers through Peyer's patches is an important uptake pathway. We report Rifampicin Lipid-Polymer hybrid nanoparticles (RIF-LIPOMER) using glyceryl monostearate as lipid and the mucoadhesive polymer, Gantrez, with the objective of balancing hydrophobicity and mucoadhesion for enhanced Peyer's patch uptake. RIF-LIPOMER was optimized for size, hydrophobicity, and mucoadhesion using Box-Behnken. Designed RIF-LIPOMER (RIF-LIPO-120) exhibited average particle size in the range 300–400 nm with drug loading > 12%. DSC and XRD confirmed complete amorphization. Contact angle and mucoadhesion force revealed that RIF-LIPO-120 exhibited greater hydrophobicity and lower mucoadhesion compared to Gantrez nanoparticles (RIF-GzNP). Comparative uptake of fluorescent labelled RIF-LIPO-120 and RIF-GzNP, through Peyer's patch following intraduodenal administration in rats, revealed the high accumulation of RIF-GzNP at the villi border, and high Peyer's patch uptake of RIF-LIPO-120. Furthermore, lower accumulation of RIF-LIPO-120 in the liver, compared to RIF-GzNP, suggested bypass of the portal circulation and lymphatic uptake through Peyer's patches. Significantly higher lung: plasma concentration ratio exhibited by RIF-LIPO-120 compared to RIF-GzNP confirmed the same ($p < 0.05$). Our study demonstrated that optimization of hydrophobicity and mucoadhesion of nanoparticles could favor Peyer's patch uptake, which in turn could enable enhanced drug accumulation in the lungs with advantage in the therapy of pulmonary afflictions.

1. Introduction

Despite decades of research and availability of highly effective treatment regimens, tuberculosis with a global kill of more than 1.5 million people (WHO, 2016), is ranked alongside HIV as the leading cause of death from an infectious disease. Pulmonary tuberculosis is the most prevalent, is primarily responsible for transmission of the disease (Pieters, 2008). Therapy with current drugs is often limited by severe hepatotoxicity, furthermore, the emergence of resistant tuberculosis, significantly contributed by patient incompliance poses additional challenges (van Hest et al., 2004). Nanotechnology-enabled targeted delivery of antitubercular drugs to lungs could play a key role in the effective management of TB, through enhanced efficacy cum reduction of off-target toxicities.

Subcutaneous injection of Poly Lactic-co-Glycolic Acid (PLGA) nanoparticles comprising anti-TB drug combinations showed sustained therapeutic blood plasma concentrations for 32 days (Pandey and Khuller, 2004a). Complete sterilization of mice lung infected with M-TB is demonstrated with Rifampicin (RIF) alone as microsphere formulation administered by intravenous route (Quenelle et al., 1999). Nevertheless, considering the current long-term treatment practiced, parenteral delivery does not appear a practical approach. Targeting drug loaded nanoparticles directly to the lungs by pulmonary administration, although a promising approach, is faced with numerous challenges (Ahmad et al., 2005). The need for large doses, difficulties in predictable dose delivery and the implications of chronic administration associated lung toxicity prove as limitations.

Oral administration of a three-drug combination of isoniazid, rifampicin, and pyrazinamide loaded alginate and chitosan microparticles demonstrated enhanced efficacy in a preclinical study (Ain et al., 2003; Pandey and Khuller, 2004b). A single administration of PLG nanoparticle comprising a four-drug combination of RIF, isoniazid, pyrazinamide and ethambutol enabled complete eradication of TB in mice and guinea pigs (Pandey et al., 2006; Sharma et al., 2004). Such high efficacy following oral administration of microparticles is attributed to transcytosis of intact nanoparticles across the gastrointestinal epithelium, via the lymphatic system through M cells and Peyer's patches (Florence, 2004; Florence and Hussain, 2001; Sanders and

* Corresponding author.
E-mail addresses: pvdevarajan@gmail.com, pv.devarajan@ictmumbai.edu.in (P.V. Devarajan).

http://dx.doi.org/10.1016/j.ijpharm.2017.09.040
Received 24 May 2017; Received in revised form 28 August 2017; Accepted 16 September 2017
Available online 19 September 2017

Ashworth, 1961). Such lymphatic absorption of micro/nanoparticles by facilitating bypass of portal circulation can enable high lung accumulation (Patel et al., 2014). Furthermore, as the initial dissemination and spread of TB bacteria occur via lymphatic drainage, the resulting high drug localization of the drug-loaded particles in the lymph could provide added therapeutic advantage.

Particle properties significantly influence Peyer's patch uptake (Desai et al., 1996; Florence et al., 1997, 2004; Thomas et al., 2011). Nanocarriers, in the size range 20 nm to 10 μm, administered orally are preferentially taken up intact through Peyer's patches (Dembri et al., 2001; O'Driscoll, 2002). However, while microparticles localized in the Peyer's patch tissue for prolonged periods, nanoparticles readily translocated systemically to various organ systems (Desai et al., 1996; Eldridge et al., 1990). Hydrophobicity favors higher uptake through M cells and hence is a critical particle property (Behrens et al., 2002; Eldridge et al., 1990). Uptake of orally administered hydrophobic polystyrene particles by the Peyer's patches and their translocation to lymph nodes and lymph vessels escaping portal circulation is demonstrated (Jani et al., 1990; Sanders and Ashworth, 1961). Solid lipid nanoparticles of hydrophobic stearic acid revealed enhanced uptake through Peyer's patches (Bargoni et al., 2001). The higher uptake of PLGA particles grafted with gelatin or chitosan as a mucoadhesive polymer through Peyer's patches confirmed mucoadhesion as yet an important feature of particulate systems for Peyer's patch uptake (Jung et al., 2000; Kawashima et al., 2000). Mucoadhesive chitosan nanoparticles due to electrostatic interaction showed higher association and internalization in Peyer's patches (Kawashima et al., 2002). Gantrez nanoparticles demonstrated good mucoadhesion and high affinity to Peyer's patches (Arbos et al., 2003, 2004; D'Souza and Devarajan, 2016; Irache et al., 2005). Higher bioavailability and lung accumulation of orally administered mucoadhesive RIF Gantrez nanoparticles (RIF-GzNP) is also reported (Date et al., 2013; Patel et al., 2014). We conjectured that association of a hydrophobic lipid with the mucoadhesive polymer Gantrez (LIPOMER) through the balance of hydrophobicity and mucoadhesion could further enhance nanoparticles uptake through Peyer's patches. Furthermore, LIPOMER could provide high drug loading of both hydrophilic and hydrophobic drugs (Benival and Devarajan, 2012).

Hence the aim of the present study was the design of RIF-LIPOMER comprising glyceryl monostearate (GMS) as lipid, and Gantrez as a mucoadhesive polymer with optimal size, hydrophobicity, and mucoadhesion by the QbD approach. The specific objective of our study was a comparative evaluation of RIF-LIPOMER with RIF-GzNP to confirm enhanced Peyer's patch uptake.

2. Materials and methods

2.1. Materials

RIF (Maneesh Pharma, Mumbai, India), Trehalose 100 (Hayashibara Co. Ltd., Japan/Gangwal Chemicals Pvt. Ltd. Mumbai, India), and Gantrez®AN-119, (Anshul Agencies, India) were received as gift samples. Gelol (Glyceryl monostearate, GMS) of Gatefosse and polyvinyl alcohol (PVA) were gifted by Colorcon Asia. Dioctyl sodium sulphosuccinate AR (Docusate sodium, Aerosol OT, AOT), and tetrahydrofuran AR (THF) and Isopropyl alcohol AR (IPA) were procured from S.D. Fine-Chem Limited (India). Mucin from the porcine stomach, Type II, (CAS-84082-64-4) was purchased from Sigma-Aldrich (USA). Doubled distilled water filtered through 0.22 μ membrane filter was used for the preparation of nanoparticles. All other chemicals and solvents were either spectroscopic or analytical grade.

2.2. Fabrication of Rifampicin LIPOMER (RIF-LIPOMER)

RIF, Gantrez, and GMS were dissolved in 10 mL THF: IPA (8:2). The non-solvent phase comprised a solution of AOT and PVA in filtered distilled water (30 mL). The organic phase was dropwise introduced to the non-solvent phase under magnetic stirring. This was followed by addition of 5% w/v magnesium acetate solution. The organic phase was allowed to evaporate completely under magnetic stirring for 6 h and the resultant nanoparticle dispersion was centrifuged and washed twice. The supernatants were pooled and used for evaluating entrapment efficiency (EE). The nanoparticles pellet was dispersed in 10 mL distilled water and probe sonicated for 5 min (5 min on/10 min off cycle) on an ice bath and particle size was determined.

For Peyer's patch uptake study, RIF-GzNP (only Gantrez without GMS) and RIF LIPOMER were loaded with Coumarin as fluorescence marker. Coumarin (2 mg) was dissolved in the organic phase prior to nanoparticle preparation. The RIF nanoparticles dispersions were freeze dried using trehalose (5%w/v) as a cryoprotectant (4.5 FREEZONE, USA). The freeze-dried nanoparticles were analyzed for particle size and drug loading. For mucoadhesion and hydrophobicity evaluation, the nanoparticles were freeze dried without trehalose.

2.3. Box–Behnken design and optimization by desirability function

Box–Behnken design was applied for the optimization of RIF nanoparticles formulation. The critical material attributes namely amount of Gantrez (X1) and concentration of AOT (X2) and the critical process parameter stirring rate (X3) were retained as the independent variables based on previous studies conducted by our group and publications (Arbos et al., 2003; Benival and Devarajan, 2012; Patel et al., 2014). The low, middle and high levels of these factors were designated as −1,0 and +1, respectively (Table 1). The response variables (dependent) were average particle size (Y1), EE (Y2), hydrophobicity (Y3) and mucoadhesion (Y4). The organic to non-solvent phase ratio (1:3), Organic phase composition (THF: IPA, 4:1), total concentration of Gantrez and GMS in the organic phase (3% w/v), RIF concentration in organic phase (1% w/v), concentration of PVA in non-solvent phase (0.3% w/v) were maintained constant.

Design Expert software (version 9.0.8) was used to generate a randomized design matrix for statistical analyses. The experimental design comprising three independent variables (X1, X2, and X3) generated 17 runs (Table 2). The formulations were run in triplicate. Based on the polynomial equation and response surface graphs, generated from outcomes of the dependent variables, the formulation, and process parameters were optimized using mathematic model. Further to validate the design model, three compositions namely, LIPO-120, LIPO-150, and LIPO-180, were generated using desirability function and the dependent variables were predicted as shown in Table 3. The generated independent variables were subsequently used to obtain the composition for optimized RIF-LIPOMER.

2.4. Evaluation of RIF nanoparticles

2.4.1. Particle size

Particle size was determined by Dynamic Light Scattering (DLS) at 25 °C using NanoBrook 90Plus PALS Particle Size Analyzer (Brookhaven Instruments, USA). The nanoparticle dispersions were appropriately diluted with filtered distilled water (0.22 μ membrane

Table 1
The Factors and Their Levels Used in Box–Behnken Design.

Factors	Level		
	Low (−1)	Middle (0)	High (+1)
X1: Amount of Gantrez AN-119 (mg)	60	120	180
X2: Concentration of Docusate sodium (AOT) (%w/v)	0	0.1	0.2
X3: Stirring rate (rpm)	750	1000	1250