PLGA Loaded Rifampicin for Treat Diverse Mycobacterial Infections

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Abstract- The aim of the present work was to minimize or prevent the degradation of rifampicin, the antitubercular drug in gastric pH condition to improve the stability and therapeutic efficacy of the drug. The study was carried out by preparing Rifampicin loaded PLGA nanoparticles using ascorbic acid as an antioxidant. Dug loaded nanoparticles were fabricated by a multistep emulsion procedure and evaluations of the prepared nanoparticles were then carried out by various methods. In this study four types of formulations were prepared. Formulation 1 (F1) is rifampicin alone loaded PLGA nanoparticles, formulation II (F2) is rifampicin – ascorbic acid (1:1) loaded PLGA nanoparticles, formulation III (F3) is rifampicin - ascorbic acid (1:2) loaded PLGA nanoparticles and formulation IV (F4) is rifampicin – ascorbic acid (1:3) loaded PLGA nanoparticles. The study concluded that ascorbic acid can minimize the degradation of rifampicin in acidic pH condition and thus improves the stability and bioavailability of rifampicin. The results also demonstrate that there is a statistically significant change in the percentage drug degradation profile when the concentration of ascorbic acid was increased.

Keyword: PLGA, Nanoparticles, Antitubercular, Rifampicin, Ascorbic Acid, Bioavailability

INTRODUCTION

Tuberculosis TB is a ubiquitous, high contagious chronic granulomatous bacterial infection caused by the Mycobacterium tuberculosis. Tuberculosis affects one third of the world population, i.e. nearly 2 billion individuals, also responsible for 3 million death annually. India accounts for 20% of all new TB cases in the world each year. [1]

The anti-TB drugs are mainly categorized into two types namely, first line and second line drugs. First line drugs include Rifampicin (RIF), Isoniazid (INH), Pyrazinamide (PYZ), Ethambutol (ETB) and Streptomycin and second line drugs include Ciprofloxacin, Levofloxacin, Ofloxacin, Saprofloxacin, Capreomycin, Anamycin, Ethionamide, Para-amino salicylic acid, Cyclosporine and Thiacteazone.

Rifampicin is a semi synthetic macrocyclic antibiotic derived from streptomyces mediterranei which has a unique role in killing the semi-dormant tubercle bacilli. Rifampicin acts by inhibiting mycobacterial DNA-dependent RNA polymerase synthesis by blocking RNA transcription. It is also known to have cytochrome P450 activity.2 RIF can be hydrolyzed to even less soluble form such as 1-amino-4- methyl piperazine under acid gastric conditions. At pH-values between 7.4 and 8.2, the molecule is oxidized to an insoluble quinone derivative or a desacetylated form. The major degradation products of rifampicin are 3-formylrifamycin, rifampicin Noxide, 25-desacetyl rifampicin and rifampicin quinine.[2]

Small molecular weight antioxidants like Vitamin C and Vitamin E plays an important role in protecting the human tissues from oxidative damage by a variety of mechanisms. Vitamin C supplements have been shown to alter many different indexes of human immune responses and the concentration of vitamin C is high in activated neutrophils and macrophages. Therefore Vitamin supplementation may prove to be beneficial.[3]

Rifampicin is well absorbed in the pH range of 1-2 even though it undergoes degradation in the acidic medium. To prevent this degradation Vitamin C can be incorporated into rifampicin-PLGA nanoparticles as an antioxidant to increase the stability of rifampicin. For a drug molecule to reach the target site from the site of administration in sufficient concentration and to maintain therapeutic levels for a sufficient period of time, a delivery system is needed. Among the various colloidal drug delivery systems available, nanoparticles represent a very promising approach to

this aim. Nanoparticles are desirable for drug delivery because of number of properties. They are known to cross the intestinal permeability barriers directly via transcellular/paracellular pathways, which explain better delivery of the encapsulated drug into the circulation.5 . In this case, nanoparticles are expected to penetrate inside the infected cell where TB is an intracellular infection.[4]

Among the various polymers used in drug delivery research, PLGA (poly-d,l-lactide-co- glycolide) is one of the most successfully used biodegradable nanosystem for the development of nanomedicines since it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid. Since the body can effectively deal with these two monomers, there is very minimal systemic toxicity associated with this polymer.

Thus the purpose of the present study was to prepare and evaluate Rifampicin loadedPLGA nanoparticles and an attempt was made to investigate the influence of ascorbic acid as an antioxidant on stabilizing rifampicin in the gastric environment by In vitro study.

Tuberculosis infection is caused by tubercle bacilli, which belong to the genus Mycobacterium. These form a large group, but only three relatives are obligate parasites, that can cause tuberculosis disease. They are part of the Mycobacterium tuberculosis complex and include M.tuberculosis, M.bovis and M.africanum. However generally only the first two are found in isolates from people with tuberculosis diagnosed in the U.K, with M.tuberculosis accounting for over 98% of isolates.6

METHODOLOGY

PREPARATION OF NANOPARTICLES

Rifampicin and ascorbic acid loaded PLGA nanoparticles were fabricated by an Emulsification/solvent evaporation method, which involved the formation of stable emulsion and evaporation of organic solvent by continuous stirring. The study was carried out by preparing four types of formulations.

Formulation 1 (F1) is rifampicin alone loaded PLGA nanoparticles, formulation II (F2) is rifampicin – ascorbic acid (1:1) loaded PLGA nanoparticles, formulation III (F3) is rifampicin -ascorbic acid (1:2) nanoparticles and formulation IV (F4) is rifampicin – ascorbic acid (1:3) loaded nanoparticles. In all the cases, drug: polymer ratio was taken as1:1 and ascorbic acid was taken in three different ratios as shown in Table 1

Procedure:

Drug loaded PLGA nanoparticles were prepared by a multistep emulsion procedure.50 mg of rifampicin and required quantities of ascorbic acid were accurately weighed and added to 10ml of dichloromethane containing the polymer [drug: polymer ratio was taken as (1:1)]. Distilled water was emulsified in the DCM containing drug and polymer to form w/o primary emulsion. It was then emulsified by sonication for 15 minutes. Primary emulsion was then poured into 8ml of 1%w/v aqueous Poly Vinyl Alcohol solution and stirred using a magnetic stirrer to form the second w/o/w multiple emulsion. The latter was then stirred continuously overnight for the complete removal DCM. The nanoparticles were then recovered by centrifugation (9000 -10,000 rpm for 15 minutes), washed thrice with distilled water and vaccum dried.

Table 1

FORMULATION CODE	INGREDIENTS
F0	PURE RIFAMPICIN
F1	RIFAMPICIN+PLGA (1:1)
F2	RIF+PLGA+ASC (1:1:1)
F3	RIF+PLGA+ASC (1:1:2)
F4	RIF+PLGA+ASC (1:1:3)

EVALUATION OF THE PREPARED NANOPARTICLES

Characterization of the prepared nanoparticles was then carried out. It includes determination of particle size, size distribution, shape, surface morphology, Poly dispersity Index and zeta potential. Scanning electron microscopy was used to determine the shape and surface morphology of the nanoparticles. Average particle size and polydispersity index of nanoparticles were measured by Laser light scattering method. Zeta potential of the nanoparticles was determined using a zetasizer.

Shape and surface morphology of nanoparticles

The morphology of Rifampicin –ascrbic acid loaded PLGA nanoparticles were analyzed using a scanning electron microscope. Samples were prepared from dilutions in distilled water of particle suspensions and dropped onto stubs using double sided sticking tape.

After air drying, particle were coated with a thin layer of platinum film and then examined by scanning electron microscopy.

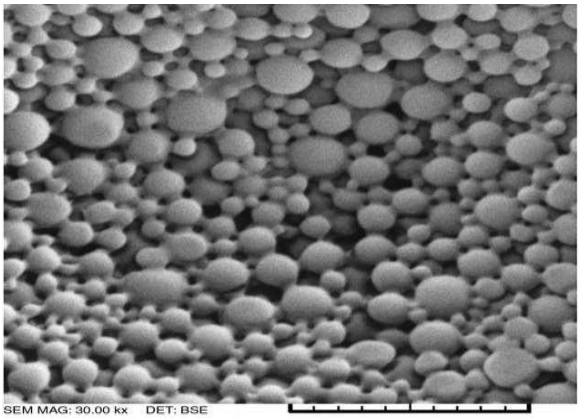
Particle size characterization of the nanoparticles
The particle size, size distribution and poly dispersity index of the nanoparticles were measured by a laser particle size analyzer after suitable dilutions.

Zeta Potential Study
The surface charge of nanoparticles was determined by the electrophoretic mobility of nanoparticles in a U type tube at 25˚C, using a zetasizer.

RESULTS

SHAPE AND SURFACE CHARACTERIZATION OF THE PREPARED NANOPARTICLES [SEM]

Scanning electron micrograph of the prepared Rifampicin - Ascorbic acid loaded PLG Ananoparticles are shown in Figure 3
SEM images revealed that the nanoparticles were spherical with smooth surface and theyare relatively mono dispersed.



2µm

PARTICLE SIZE CHARACTERIZATION OF THE NANOPARTICLES
The mean particle size and polydispersity Index of all the samples were determined

S.NO	FORMULATIONS	MEAN DIAMETER (nm)±SD	PdI
1.	RIFAMPICIN+PLGA(1:1)	375± 20	0.312
2.	RIF+PLGA+ASC(1:1)	378 ± 22	0.316
3.	RIF+PLGA+ASC(1:2)	374 ± 18	0.308
4.	RIF+PLGA+ASC(1:3)	380 ± 23	0.317

Laser particle size analyzer yields the diameter of the bulk population. Particles were in the size range of 374-380 ± 18-23 (SD) nm. Polydispersity index is a measure of the distribution of particles in a given polymer sample. It gives the distribution range from 0.000 to 0.500. Polydispersity index greater than 0.5 indicates aggregation of particles. Here it is in the range of 0.308 - 0.317.

ZETA POTENTIAL STUDY
Zeta potential is a term related to the stability of samples. For molecules and particles that are small enough, high zeta potential will confer stability i.e. it resist aggregation. Here zeta potential of the prepared nanoparticle was found to be -46.6, which would not allow aggregation.

SUMMARY & CONCLUSION

The results of the study demonstrate that ascorbic acid can minimize the degradation of rifampicin in gastric pH condition and thus improves the stability and therapeutic efficacy of rifampicin. The study also concluded that there is statistically a significant change in the percentage drug degradation profile when the concentration of ascorbic acid was increased. Further invivo studies are recommended to address the therapeutic efficacy of rifampicin –ascorbic acid loaded PLGA nanoparticles.

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