

**FORMULATION DEVELOPMENT & EVALUATION OF  
RIFAMPICIN NIOSOME FOR ANTI-TUBERCULAR TREATMENT  
WITH ISONIAZID**

**SYNOPSIS**

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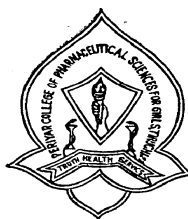
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## Introduction

With approximately 3 million annual deaths in the 1990s, tuberculosis remains a leading cause of mortality worldwide into the 21st century. It is estimated that one-third of the world population harbour a latent infection by the causative pathogen, *Mycobacterium tuberculosis* (*M. tuberculosis*). One of the hallmarks of tuberculosis (TB) is the persistent phase of infection. During this phase the bacteria are thought to be in a slow growing or non-growing state and are recalcitrant to treatment by conventional anti-TB drugs (Smith *et al.*, 2004). One of the drawbacks of existing TB drugs is that they target actively growing bacteria in cell processes such as cell wall biogenesis and chromosome replication.

Patients who carry a latent infection are at risk of reactivation of this disease and this factor which causes a major obstacle to the global control of TB. To treat an infection, a cocktail of drugs including, for example, isoniazid, rifampicin, ethambutol and pyrazinamide are prescribed for 2 months followed by a continuation phase in which isoniazid and rifampicin are taken. Long-term therapies lasting between 6 and 9 months have frequently led to patient non-compliance and, in turn, contributed to the emergence of multi-drug resistant TB (MDR-TB). MDR strains, such as the notorious Strain W, are increasingly being found which are resistant to many first-line agents including isoniazid, rifampicin, ethambutol, streptomycin and pyrazinamide, as well as some of the second-line drugs, such as ethionamide, cycloserine, thiacetazone and the quinolone derivatives. The cost of treating a patient carrying MDR-TB is much greater, typically running into tens of thousands of dollars per patient, than for patients carrying a drug-sensitive strain. Without effective treatments, the fear is that the number of infections caused by MDR-TB will increase out of control.

Even though a vaccine and numerous effective antimycobacterial agents are available for its treatment, several million people die from the disease each year (Brennan PJ 2003). An important consideration in the treatment of tuberculosis is the fact that the etiological agent, *M. tuberculosis*, has the ability to persist intracellularly in the host macrophage for long periods of time (Collins FM 1998). The course of treatment is, therefore, dependent upon the intracellular delivery of antimycobacterial agents for prolonged periods. This becomes even more important when one considers the ability

of *M. Tuberculosis* to persist in a dormant state, thus giving rise to a large group of infected individuals who carry the organism in a subclinical state without having active disease. It has been estimated that about 30% of Indians are infected and at risk of development of active disease. Worldwide, it is estimated that one-third of the population is infected with *M. Tuberculosis*, which results in about 8 million new cases of tuberculosis annually (Centre for disease control and prevention 2000).

Properly devised delivery techniques should theoretically circumvent these problems by positioning effective drugs within host macrophages, thus giving direct access to dormant organisms that presumably would be within macrophages or in the surrounding lymphatic area (Wright EL *et al.*, 1996). In the case of a drug that is effective against actively multiplying *mycobacteria*, this would be advantageous because the drug would continually be available for prolonged periods at the site in the event the organism underwent any multiplication cycle. Niosome technology has the capability of accomplishing these goals by achieving intracellular delivery of antimycobacterial drugs and allowing programmed controlled release over a prolonged period (Aliasgar S and Misra A 2002).

The niosome formulations used in the present study are known to be biocompatible and capable of degradation to lactic and glycolic acids by non-enzymatic reactions (Aarthi J and Deepthi I 2001; Abazinge MT *et al.*, 2000). This technology has been used for sustained delivery of various biological components, including antigens, steroids, peptides, proteins, and antibiotics.

**Aim**

The aim is to find a dosage regimen for the treatment of tuberculosis using sustained dosage of rifampicin loaded in niosome vesicles administered intraperitoneally and oral dosing of isoniazid. Formulations were developed in such a way as (i) to provide sustained programmed release of the drugs in order to circumvent the multiple dosing required for conventional therapy and (ii) to provide a means of delivery of the drugs to the macrophages where *mycobacteria* reside during an infection (Aarthi J and Deepthi I 2001; Abazinge MT *et al.*, 2000 and Agnishwar G 2006).

Rifampicin and isoniazid were both first-line drugs used in the therapy of tuberculosis and are included in the list of recommended drug regimens for treatment of latent *M. Tuberculosis* infection in adults (Ahmed SG *et al.*, 2005 and Aliasgar S and Misra A 2002). They have been used in combination for treatment of tuberculosis in clinical trials of human immunodeficiency virus-negative and human immunodeficiency virus-positive persons. As a part of this study, the use of rifampicin-loaded niosomes in a combined therapeutic regimen with oral dosages of isoniazid were studied and evaluated.

## Literature Review

Management of tuberculosis continued to remain a challenge owing to poor bioavailability of anti-tuberculous drug or bacterial resistance. Different strategies have been attempted by researchers.

Alisgar S and Misra A (2002) reported a niosome based transdermal drug delivery system of nimesulide which was developed and extensively characterized and evaluated for *in-vitro* performance followed by *in-vivo* evaluation in rats by carrageenan induced rat paw oedema method. The finding of this investigation conclusively demonstrated the prolongation of drug release and increase in amount of drug retention into the skin and permeation across the skin after niosomal encapsulation of nimesulide. They concluded that the nimesulide niosomal gel formulation which was developed had also enhanced anti-inflammatory activity compared to plain drug gel and marketed formulation.

Chandraprakash KS *et al.*, (1993) showed that the synthetic analogues of liposomes prepared from non-ionic surfactants, known as niosomes, had been used as vesicular drug carriers. Diclofenac sodium had been entrapped in niosomes comprising Tween 85 and Tween 85-poloxamer F 108 mixture. Anti-inflammatory efficacy of these niosomes was compared with that of free diclofenac sodium in adjuvant induced arthritic rats. It was found that niosomal diclofenac sodium formulations prepared by employing a 1:1 combination of Tween 85 and poloxamer F 108 elicited a better and consistent anti-inflammatory activity for more than 72 hours after administration of a single dose.

Oommen E *et al.*, (1999) explained about the anticancer therapy could be made more effective by targeting the delivery of anticancer drugs to the tumour site more quantitatively. Niosome encapsulated bleomycin and thermosensitive niosomal bleomycin were prepared by lipid layer hydration method. The antitumour efficacy was assessed using two tumour models viz. Sarcoma-180 and Ehrlich ascites using Balb/C mice. Accumulation of higher bleomycin levels after macrophage activation exerted increased antitumor effect. This present study suggested that a more

quantitative delivery of bleomycin encapsulated in niosomes, to the tumour site was possible after macrophage activation.

Mullaicharam AR and Murthy RSR (2004) reported a niosomal drug delivery system of rifampicin, widely used first-line anti-tuberculosis drug, was developed using factorial design and the formulation procedure along with the drug entrapment efficiency of the niosomes were optimized. The prepared niosomes were characterized for size, shape and lamellarity. The stability of niosomes in terms of retention of drug was measured at refrigerated temperature (5°C) and ambient temperature (25-35°C) for the period of 60 days. They concluded that the developed formulation might be useful in the treatment of pulmonary tuberculosis.

Wright EL *et al.*, (1996) demonstrated the potential of encapsulating all transretinoic acid (ATRA) in niosomes and delivering it as an inhaled aerosol. Niosomes might provide a means to reduce the toxicity of ATRA and alter the pharmacokinetics in a manner similar to liposomes. Various non-ionic surfactants were used to achieve optimum encapsulation and nebulization efficiencies, and the best formulations were obtained with combinations of (Span 20 + Tween 80) and (Span 60 + Tween 80) using an ATRA concentration of 1 µg/ml. The aerosol produced with the selected niosomal formulations upon nebulization in PARI LC Star nebulizers driven by a pulmo-aide compressor. The results of this work were very encouraging and offer an alternative approach to the respiratory delivers of ATRA by aerosolization.

Carafa M *et al.*, (2002) reported the topical application of lidocaine-loaded (LID) non-ionic surfactant vesicles (NSVs) was prompted by the great interest on new delivery systems for local anaesthetics. NSVs were prepared from Tween 20 and cholesterol. The effect of vesicle composition and environmental pH condition (8.6 –5.5) on drug encapsulation efficiency was investigated. Diffusion experiments showed that the flux of charged lidocaine through silastic membrane was possible only after the vesicle encapsulation. Permeation through mouse abdominal skin of LID HCl loaded vesicles showed a higher flux and a similar lag time with respect to classical liposome formulations, while LID permeation rate was quite similar for NSV and liposome formulations.

Elizabetta G *et al.*, (1997) demonstrated to prepare niosomes which have high encapsulation capacity for soluble drugs, starting from span 60 and cholesterol, an

improved method, evaporation – sonication method was proposed. The corresponding niosomes showed a good stability at least 40 days. Colchicine was chosen as a model drug for examining the capsulation capacity of these niosomes. The results demonstrated that niosomes prepared in this way not only had high encapsulation capacity, but also reduced the side effects of drugs.

Joseph *et al.*, (2003) demonstrated the potential of niosomes with a versatile anticancer drug were prepared by ether injection technique using surfactant (Tween 40 or 80), cholesterol and drug in 4 different ratios. The niosomes were characterized for size, shape, entrapment, efficiency, stability and *in vitro* release profile. The ideal batch was evaluated for further *in-vivo* anti-tumour efficiency study in Swiss albino mice. The results revealed that the niosomes formulated had the potential to produce a significant anti-tumour effect than the free drug in terms of higher percentage increase in life span and increase in percentage tumour cell inhibition.

Ramesh Panchagnula *et al.*, (2004) showed that the tuberculosis (TB) needed treatment with three to five different drugs simultaneously, depending on the patient category. These drugs could be given as single drug preparations or fixed dose combinations of two or more drugs in a single formulation. The study was designed to be an open, crossover experiment. A total of nine blood samples each of 3 ml volume were collected over a period of 24 h. The concentration of rifampicin, its main metabolite desacetyl rifampicin (DRIF), isoniazid and pyrazinamide in plasma were assessed by HPLC analysis. It was concluded that four drugs FDC tablet is bioequivalent for rifampicin, isoniazid and pyrazinamide to separate formulation at the same dose levels.

Agrawal S *et al.*, (2001) explained that depending on the patient category, tuberculosis required treatment with 3 to 5 drugs which means that patient's compliance to therapy might not be optimal. To increase patient's adherence to treatment schedules, these drugs can be given as single drug preparations or fixed dose combinations of 2 or more drugs in a single formulation. However, an important issue associated with rifampicin containing FDC is its quality. They concluded that the FDC tablet containing 4 drugs is bioequivalent to separate rifampicin, isoniazid and pyrazinamide formulations at the same dose levels.



Barrow ELW *et al.*, (1998) reported on the use of Rifampicin-loaded microspheres to effectively treat *Mycobacterium tuberculosis*-infected macrophages and mice. Using similar biocompatible polymeric excipients of lactide and glycolide copolymers, they have increased the rifampicin loading of small microsphere formulations (1 to 10  $\mu\text{m}$ ) by four fold. The results demonstrated the ability to use small microsphere formulations alone to achieve significant results in a murine tuberculosis model and also the ability to use them safely in combination with another antimycobacterial agent.

Surez S *et al.*, (2001) reported that a *Mycobacterium tuberculosis* (H37Rv)-infected guinea pig model was used to screen for targeted delivery to the lungs by insufflation (with lactose excipient) or nebulization, of either rifampicin alone, rifampicin within poly(lactide-co-glycolide) microspheres (R-PLGA) or polymer microparticles alone (PLGA). Animals treated with single and double doses of R-PLGA microspheres exhibited significantly reduced numbers of viable bacteria, inflammation and lung damage compared with lactose-, PLGA- or rifampicin-treated animals 28 days post-infection ( $P < 0.05$ ). Two doses of R-PLGA resulted in reduced spleen enlargement. These studies support that the potential of R-PLGA delivered to the lung to treat pulmonary tuberculosis.

Gong LK *et al.*, (2005) reported that the current therapeutic approaches for pulmonary fibrosis, which was characterized by fibroblast proliferation and extracellular matrix remodeling, were unsatisfactory. Feitai, consisting of several herbs, was a folk formula for pulmonary tuberculosis therapy in China. To investigate the effects of Feitai on pulmonary fibrosis, Feitai was administered orally to bleomycin (BLM)-treated rats, and the lung toxicity effects were evaluated according to inflammatory cell count, protein concentration, and lactate dehydrogenase (LDH) activity in the bronchoalveolar lavage fluid (BALF), malondialdehyde level and hydroxyproline content in lung tissue 28 days post-BLM. Serial sections of the lung were stained with hematoxylin and eosin (HE) and Masson trichrome, respectively. The degree of fibrosis was assessed quantitatively using *LEICA* QWin image analyzer. Results showed that Feitai inhibited BLM-induced lung fibrotic lesions in a dose-dependent manner as reflected by decreased the lung hydroxyproline content and lung fibrosis fraction 28 days after BLM instillation. Treatment with Feitai also significantly

ameliorated the BLM-induced lung toxicity effects detected in BALF and lung tissue. The effects *in-vitro* on WI-38 human lung fibroblast cell line showed that Feitai significantly reduced the cell proliferation and transforming growth factor (TGF)- $\beta$  stimulated type I collagen synthesis. These results strongly demonstrated that Feitai might be useful in the treatment of pulmonary fibrosis.

The foregoing review of literature indicates that there had been considerable work reported on beneficial effects of niosome technology in alleviating the disease conditions effectively. Literatures were also available on application of niosome technology to few antituberculous drugs and their advantages in improving the current therapy of tuberculosis.

However a little progress has been made on the development of niosome of rifampicin.

To the best of our knowledge there had been little progress on the study of niosome formulation of rifampicin. Hence the present study was attempted to develop niosomes of rifampicin and optimized the formulation for effective management of tuberculosis.

## Objective

Although directly observed treatment - Short course (DOTS), contributes for the control of the tuberculosis, non compliance with treatment continues to be the main cause of poor results (Arachi A 1991 and American Thoracic Society Documents 2002). The main reason for non - compliance is the long, 6 - month duration of the treatment. Hence shortening of the duration of treatment without compromising the cure and relapse rates still remains a major goal for control policies. The drugs of choice and first line of treatment for tuberculosis are rifampicin and isoniazid. By using these two drugs with different approach, it is presumed that the dose level of these two drugs will be reduced and also the time period of treatment.

Another difficulty with the treatment of the tuberculosis is Multidrug Resistant – Tuberculosis i.e. MDR-TB (Sharma SK 2004). With this approach of reduced dose and duration, it is possible to enhance chemotherapy and more importantly improve patient compliance, which is rather a prime factor for the failure of tuberculosis treatment.

The objectives of this extended study were, therefore, to (i) perform preformulation studies of the formulations (ii) prepare niosome formulations of rifampicin using various surfactants (iii) increase the drug loading of the small niosomes, (iv) test their ability to treat an *M. Tuberculosis* infection in an animal model without the use of the larger niosome formulations, and (v) evaluate the level of activity of combined therapy with the rifampicin-loaded small niosomes and an oral regimen of another antimycobacterial drug, isoniazid, by performing histopathological studies.

## Materials & Methods

The niosomes were prepared with various ratios of surfactants, cholesterol and dicetyl phosphate. The formulation which produced better physicochemical characteristics, maximum entrapment and drug release with highest duration of release was selected for further studies. The ratio of surfactant, cholesterol and dicetyl phosphate that was employed was 47.5:47.5:5.

These ingredients were dissolved in diethyl ether (10-15 ml). The solvent was evaporated under reduced pressure at a temperature of about 60°C using a rotary flash evaporator. This temperature was achieved by rotating the round bottom flask, under reduced pressure, about 1.5 cm above a boiling water bath, leaving a thin layer of solid mixture deposited on the wall of the round bottom flask.

The 10ml of 1mg/ml solution was added to the flask heated to about 50°C on the water on a vortex, until a good dispersion of the mixture was obtained. The suspension was then sonicated for 15 minutes to form unilamellar niosomes.

Each niosome formulation was analyzed for drug content by spectrophotometric assays. The size distribution for each lot of rifampicin-loaded niosomes was determined with a Coulter LS Particle Size Analyzer (Quenelle DC *et al.*, 1999). Niosome lots were sterilized by filtration technique using Millipore filter. Following sterilization, niosome formulations were stored in desiccators at 20°C until use. The surface morphologies of niosome formulations were examined by scanning electron microscopy (SEM). Matching placebo formulations were prepared without drug and compared for each drug loaded preparation. The rifampicin content of each lot of niosomes was determined by first extracting the rifampicin from a known quantity of niosomes and quantified spectroscopically at 474 nm. The concentration of rifampicin contained in each sample was determined by measuring the absorbance on a spectrophotometer at 474 nm. Calibration curve was prepared in the concentration range from 5 µg/ml to 50 µg/ml. A series of rifampicin solutions of known concentrations in methanol were prepared, and their absorbances were measured in order to generate a standard curve. The rifampicin concentrations in the niosome and

control samples were then obtained by linear regression, and the total amount of rifampicin was calculated.

*M. Tuberculosis* H37Rv was maintained on Middlebrook 7H10 agar slants containing 0.5% glycerol and 10% oleic acid-albumin-dextrose-citrate (OADC) (Difco). The MIC of rifampicin for this strain is 0.06 to 0.25 g/ml (Berner P *et al.*, 2002).

Swiss albino mice of male sex (weight, 14 to 16 g) were maintained on a diet of Teklad sterilizable laboratory feed (Harlan) and water throughout the studies. All animal research programs and facilities in our animal house have the approval of CPCSEA of Government of India. Approval for the studies was given by the institutional animal care.

The mouse model described here was a nonlethal, short-term model that was developed in order to investigate antituberculous drugs. The inoculum size and time frame were therefore selected to ensure that death would not occur due to the large inoculum size and also that the drugs could be screened with a short turnaround time. Mice were inoculated via the lateral tail vein on day 0 with approximately 10<sup>5</sup> viable *M. Tuberculosis* H37Rv organisms in a volume of 0.1 ml of 0.9% sterile sodium chloride solution. Drug treatments were initiated approximately 4h post-inoculation. Each treatment group contained 10 mice.

Rifampicin niosomes were formulated with a concentration of 50 mg and 100 mg of rifampicin. Niosome formulations, including rifampicin-loaded and placebo niosome formulations, were injected intraperitoneally on days 0 and 7 by using 50 mg dose and 100 mg dose after suitable dilution with normal saline where the members of each group received equivalent doses of rifampicin of 3.0 and 6.0 mg per mouse. Assuming an average weight of 0.015 kg, this would be equivalent to approximately 200 and 400 mg/kg of body weight, respectively. Each injection was suspended in sterile saline by using a sterile tuberculin syringe with a 23-gauge needle (Shivani D *et al.*, 2001).

For mice receiving isoniazid, each was dosed by individual weight by a gavage technique (atraumatic stainless steel oral dosing needle attached to a sterile 1-ml syringe) with a volume of 0.1 ml/10 g of body weight. Isoniazid was dissolved in sterile water prior to oral administration. The oral gavage of isoniazid was performed daily from day 0 to day 25. All mice were weighed daily and were observed for

clinical signs of toxicity. On day 26, mice were anesthetized with ketamine-xylazine for aseptic collection of liver, spleen and lungs. The organs were removed and frozen individually in sterile bags.

The mice were treated with a combination of rifampicin-loaded niosomes and various oral regimens of isoniazid by the procedures described above for individual therapies.

The experimental protocol for the combined therapy was established.

During the efficacy studies, the levels of infection in mouse were determined for each formulation for mice receiving only the rifampicin-loaded niosome therapy. At 7, 14, and 21 days, three mice per group were anesthetized with ketamine-xylazine and organs such as liver, lungs and spleen were collected. At 26 days, all 10 mice were anesthetized with ketamine-xylazine and exsanguinated by cardiac puncture.

## Observations

Preformulation studies are integral and important one for a stable formulation.

Preformulation studies were carried out by using DSC and FT-IR.

The DSC thermograms of individual ingredients like rifampicin, cholesterol, dicetyl phosphate, span 20, span 40, span 60, span 80 and drug excipients physical mixtures were obtained and compared for the compatibility.

The drug-excipients physical mixtures and also drug- physical mixtures were obtained and compared. The results revealed that there was no interaction between the drug and other excipients used in the formulation.

The niosomes were subjected to microscopic examination in Atomic Force Microscopy and Scanning Electron Microscope for characterizing size and shape of niosomes. Microscopic examination results revealed that the optimized rifampicin niosome formulation is spherical small unilamellar vesicles of 30-300 nm.

The entrapment efficiency of rifampicin niosomes using various surfactants like Span 20, Span 40, Span 60 and Span 80 were measured. The percentage entrapment of rifampicin niosomes was found to be in the acceptable ranges.

Increasing the sonication time resulted in reduction in percent drug entrapment; the decrease in percent drug entrapment is may be due to leakage of the drug during sonication. Cholesterol provides endurance against mechanical strain during sonication and centrifugation.

Sonication brings about size reduction by breaking large niosomes into smaller ones and in doing so, leakage of small quantities of drug from the niosomes occur. Hence the sonication time was optimized to 15 min, and further reduction in the size by increasing sonication time was not attempted.

The *in vitro* release study was carried out by diffusion method using sigma dialysis membrane as a barrier. From this study, the percentage of drug diffused into the medium was evaluated. The maximum percentage of drug diffused at the maximum time from the rifampicin niosomes using various surfactants like span 20, span 40, span 60 and span 80 were obtained and found. The rifampicin niosome formulation with span 60 was found to have maximum percentage of release with maximum time

duration. The result showed that rifampicin niosomes with span 60 had highest entrapment and maximum longevity of release.

The Bactericidal Activity of the rifampicin niosome was studied by BACTEC radiometric method use in the resistant strains (RF 8554) and sensitive strains (H37RV) of *M. Tuberculosis* (Salman H *et al.*, 1981).

Rifampicin drug 5 mg/5 ml solutions were prepared in 0.5 ml of DMSO and 4.5 ml of sterile distilled water. From the stock solution 80 µg/ml & 40 µg/ml was prepared, so that 0.1 ml (8 µg/ml) corresponds to 2 µg/ml and 1 µg/ml in 4 ml of Bactec medium.

The result of Daily Growth Index (GI) readings were taken for 7 days.

The control reading of resistant strain (RF 8554) of *M. Tuberculosis* showed a marked multiplication from the 4<sup>th</sup> day onwards.

Rifampicin (1 µg) drug showed that the strain was resistant to the drug and reduced the activity by the increased GI and attained a maximum of 999 on 6<sup>th</sup> day. But in increased concentration (Rifampicin 2 µg) the reduction of the activity of drug was less and slow increased GI when compare to Rifampicin (1 µg) drug and it also attained a maximum of 999 on 6<sup>th</sup> day.

Rifampicin niosome (1 µg) showed the GI was increasing uniformly for 5 days and it reached a maximum of 999 indicating the resistance, but greater activity for niosome was shown with relatively less GI than drug alone for 5 days and reached a maximum of 999 on the 6<sup>th</sup> day, which is equal to the drug alone.

Rifampicin niosome (2 µg) showed greater activity and relatively less GI when compare to the same concentration of the drug and it has not reached the maximum resistant on 6<sup>th</sup> day. It showed a less resistant than the drug alone.

It showed that the control reading indicate the sustained multiplication over a 7-day period. The growth of *M. Tuberculosis* reached a recognized GI (Growth Index) from 6<sup>th</sup> day.

For *in-vivo* animal studies, two strengths 50 mg (5% w/w) and 100 mg (5% w/w) of rifampicin niosomes were produced and assessed. Each lot was produced in order to obtain a diameter of 30 to 300 nm. The size distributions of both lots of rifampicin-loaded niosomes demonstrated a Gaussian curve. Examination by SEM revealed no evidence of cracks, holes, or major defects in the outer films of the formulations.



In isoniazid oral therapy, the oral regimen for isoniazid consisted of the following dosages: 25, 12.5, 6.25, 3.125, and 1.56 mg/kg. Analysis of the data reveals that the effective range for absence of lesions in the lungs and spleens were observed (Orozco LC *et al.*, 1986 and Morbidity and Mortality Weekly Report 2003).

For least doses of isoniazid, a significant increase in the number of lesions was observed. This was due to the stress induced by the extra handling (i.e., daily dosing) of these treated groups compared to that required for the mice in the non-treated group, which were not handled on a daily basis.

The results of the niosomes therapy revealed that treatment with 50 mg rifampicin loaded niosomes without isoniazid (given as two separate intraperitoneal injections on days 0 and 7) did not show any reduction of lesions in the lungs or spleens at the end of 26 days. The same treatment was given with 100 mg rifampicin loaded niosomes showed some significant reduction of lesions in the lungs and spleens by the end of the 26 days. None of the placebo-treated groups showed any significant decrease in the numbers of lesions at the end of the experimental period in either the lungs or the spleens.

With the therapeutic range used in experiment for isoniazid, significant reductions in the numbers of lesions in the lungs and the spleens were observed for all dosages. Thus, it was difficult to show significant differences in the reduction of lesions with the combined niosomes therapies compared with that achieved with the oral regimen of isoniazid alone. The one exception was the isoniazid dose of 3.125 mg/kg in the spleens, for which it was possible to show a significant difference between the combined therapy with the niosomes and the oral isoniazid therapy. Both the 50 mg and 100 mg doses of niosomes resulted in significant improvements in the reduction of lesions compared with that achieved by therapy with only the oral isoniazid at 3.125 mg/kg. For the oral isoniazid therapy (3.125 mg/kg), when combined with the 50 mg or 100 mg niosomes therapy, complete elimination of lesions was obtained. With the combination of niosomes plus the higher range of oral isoniazid therapy, complete elimination of lesions from the lungs was observed for two of three combinations with the 50 mg niosome dose and three of three combinations with the 100-mg niosome dose. In the spleens, five of the six combination therapies with the

50 mg and 100 mg niosome dosages resulted in the complete elimination of lesions, something not achieved with any of the dosages of isoniazid alone.

The therapeutic dose range for isoniazid was decreased to 1.56 mg/kg in order to evaluate less effective dosages. Among the doses in this dose range, only the dose of 1.56 mg/kg was able to produce a significant reduction in the numbers of lesions in the spleens.

The stability study for the optimized formulation was carried out as per ICH guidelines ( $40 \pm 2^\circ\text{C}/\text{RH } 75\%$  for 6 months). The formulation showed no significant change with respect to physical appearance, drug content and dissolution pattern. Based on the above results it can be concluded that the prepared formulation is stable.

## Summary

From the preliminary work, it was observed that, span 60 niosomes was better than that of span 20, span 40 and span 80 niosomes. In terms of storage stability, span 60 niosomes when stored at 45 °C 75% RH, exhibited a drug leak of less than 10 % even after 6 months of storage

It was also evident that better entrapment efficiency of above 50% was achieved in this project.

*In vitro* release of the niosomes with various surfactants was studied. The niosomes prepared with span 60 gave prolonged release and more amount of drug was released than with other surfactants.

*In-vivo* characteristics of sterilized rifampicin niosomes prepared with span 60 was administered intraperitoneally along with isoniazid oral dose to mice. Isoniazid with a minimum dose of 1.56 mg/kg for 26 days orally along with minimum 50 mg dose of rifampicin niosomes on 0 and 7 days administered intraperitoneally produced excellent result in histopathological studies in liver, lungs and spleen.

## **Conclusion**

A therapeutic regimen with the rifampicin niosomes in combination with oral regimens of isoniazid was able to safely increase the effective therapeutic range of Isoniazid.

In most cases, combination therapy reduced the appearances of lesion in the lungs, spleens and liver to non detectable levels, something not achieved with the oral regimen of isoniazid alone. The results were achieved by using a much reduced dosing schedule for rifampicin. Instead of dosing the mice daily 26 days, as would be the case with an oral dose, administration of dose to the mice only twice during that period and achieve significant improvement of the isoniazid oral therapy . These findings are encouraging and demonstrative that niosome technology can be safely used in combination with another antimicrobial agent.

With the addition of the results presented in this report, it seems apparent that niosome technology can offer an alternative therapy for treatment of tuberculosis.

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