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Exploring the Use of Lipid Based Nano-Formulations for the Management of Tuberculosis

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Abstract

Tuberculosis (TB) is an airborne infectious disease spreading very fast from person to person, which affect the health as well as socioeconomic conditions harshly. Though varieties of antitubercular drugs (ATDs) are available for its treatment, associated stern side effects restrict the patients to receive complete therapeutic benefits. Moreover, emergence of drug- resistant tuberculosis and co-infection of TB with HIV further worsen the situations. Polymeric formulations are introduced for progressive and long-term delivery of therapeutic agents, but owing to their noted limitations, performance is not up to the mark. In this juxtaposition, lipid based nano-formulations are introduced as an alternative to the polymic formulations in the management of TB with an intention to overcome side effects related to drugs along with limitations of polymeric formulations. The lipid based formulations comprise nanoemulsions, solid-lipid nanoparticles (SLNs), nano-structured lipid carriers (NLCs), liposomes, and niosomal systems, etc.

Liposomes have more promising antitubercular activity as its intended for targeted drug delivery especially to the infected part. Further mannosylation of liposomes offers tremendous results in TB chemotherapy as it directly binds to mannose receptors available on the surface of alveolar macrophages resulting mycobacterium destruction. Niosomes may have superior drug targeting ability, chemical stability, osmotic activeness and *in vivo* activity in comparison to that of liposomes. SLNs and manosylated SLNs are the advanced form of the lipid formulations which enhance the drug uptake at the infected organ and show significant *in vivo* anti-tubercular activity with reduced toxicity. Moreover, NLCs shows its satisfactory potential against MTb along with drug targeting action. Advancement on the development of miscellaneous or other vesicular lipid formulations are not encouraging in the field of tubercular chemotherapy. Hence, lipid based formulations could be successfully employed for targeted delivery of ATDs with promising anti-tubercular activity.

Keywords Tuberculosis; Nano-emulsions; Niosomes; Liposomes; Koch's disease

Introduction

Tuberculosis

Pott's disease, *Phthisis pulmonalis*, Corpse disease, Yellow Emperor, The Captain of all these men of death, The King's Evil, Scrofula, The Wasting Disease, Bad Palace, Grievous Consumption, Divine Farmer, Romantic disease, Koch's disease, The Great White Plague, Tuberculosis, Multidrug-resistant tuberculosis (MDR-TB), Single drug resistant TB (SDR-TB), or Extensively drug-resistant tuberculosis (XDR-TB), whatever the specific terminology by which this infectious disease is documented over time, there is no query that tuberculosis (TB) has been coupled with, and a burden carried, throughout known history and human prehistory.

Tuberculosis (TB), found to be a common insidious airborne contagious disease worldwide, prevalent in most of the developing nations while resurgent in developed and developing nations. It puts a major strain on public health, occupying the second position following HIV/AIDS in causing high mortality rates globally [1]. TB is known to be caused by the small aerobic non-motile bacillus bacteria *Mycobacterium tuberculosis* (MTb) since 1882, when Hermann Heinrich Robert Koch, communicated the results of his studies on the tubercle bacillus to the Berlin Physiological Society and hence after named Koch's bacillus [2].

The beginning of the disease starts with the invasion of different strains of Mycobacterium, but mostly *Mycobacterium tuberculosis* into the lungs. Although the first reliable anti-tubercular drug Streptomycin discovered in 1944 by Selman Abraham Waksman, still the disease remained as the main cause of ill health and preventable death worldwide. Moreover, some of the other disease or conditions also responsible for very high susceptibility of tubercular infections, such as human immunodeficiency virus (HIV) infection, diabetes, chronic lung disease [3]. Additionally, the long-term use of corticosteroids, TNF-α blockers, polymorphisms in vitamin D receptors, malnutrition and smoking are few other factors in the increased susceptibility to tubercular infection [4]. The co-infection of HIV/AIDS with MTb is named as "Cursed Duet" which accounts for around one in three AIDS-related deaths [5]. As per WHO report, in 2014, approximately

77% HIV- positive TB patients began or went on to antiretroviral therapy [6]. Further this "Cursed Duet" threatens as a socio-economic disaster for the co-infected families as around 30% of the yearly revenue is being directly and indirectly spent by the infected family.

Moreover, Mycobacterium has worsened the problem due to the emergence of various types of resistances [7] and threatens global TB control. Multi-drug resistance (MDR) tuberculosis arises due to poor therapeutic practices with respect to the use of first-line antitubercular drugs (f ATDs) [1], whereas extensively drug-resistant TB (XDR-TB) (around 10% of MDRTB cases) arises due to the direct consequence of two most important class of Second-line antitubercular drugs (sATDs) Fluoroquinolones (FQs) and Aminoglycosides (AGs) and the recent emergence of new strains of totally drug-resistant TB in densely populated cities such as Mumbai (India) and Teheran (Iran) [8].

Epidemiology of tuberculosis

If the consequence of an ailment for mankind is estimated from the number of sufferers which are owing to it, then TB must be assumed much more significant than the most dreaded infectious diseases like plague, cholera. TB is responsible for one out of seven human death proved statistically and this was what Robert Koch mentioned in his famous published lecture in the Berliner Medicinische Wochenschrift, under the title "Die Aetiologie der Tuberculose" on April 1882 [9] which signifies its harshness during that time. TB is a pernicious disease and based on this old scourge's grievousness, the World Health Organization (WHO) declared it as a global public emergency in 1993 [1].

It is realized that 134 years after the discovery of the tubercle bacillus, 64 years after the discovery of Streptomycin(s), and billions of dollars spent by various organizations and governments of all countries throughout the globe every year still it remains out of the bound and peoples are dying from this curable lethal infectious respiratory disorder. To know the reason we must and should consider the TB epidemiology and the data are summarized in Table 1. MTb mainly finds its fatalities in developing nations where social and health situations are ruined and the access to medicines is limited.

Moreover, the incidence of some associated provisions compromising the immune system functionality, such as alcoholism and HIV infection favours break through the infection [3] and makes the treatment more complicated. Finally, the course of therapy and its long period, particularly in the case of resistant TB, further complicate the scenario. Now it is easy to understand why many people still die from the MTb infection. We should not criticize and distrust on therapeutic efficacy, as first of all TB is a social disease [10]. Despite all the advances made in the treatment and management, this old scourge still considered as one of the key public health problems that have plagued mankind for millennia.

TB	Category	2014 (In Million)	2013 (In Million)	2012 (In Million)
New TB Case	Male	5.4	5.15	5.17
	Female	3.2	3.3	2.9
	Child	1.0	0.55	0.53
	Total	9.6	9.0	8.6
	HIV +ve	1.15	1.1	1.1
	HIV -ve	8.45	7.9	7.5
TB Death	Male	0.89	0.91	0.82
	Female	0.48	0.51	0.41
	Child	0.14	0.08	0.07
	Total	1.5	1.5	1.3
	HIV +ve	0.4	0.36	0.3
	HIV -ve	1.1	1.14	1.0

Table 1: WHO's global tuberculosis status 2012-14.

Pathophysiology of tuberculosis

"All is decided the first day, which gets the longest day", a famous quote of de Martino et al., which was meant to describe the initial combat of TB once it invades the host [11]. Though various species of mycobacteria are plentily available in soil and water but *M. tuberculosis* along with seven other very close myco-bacterial species are recognized as the *M. tuberculosis* complex- MTb possesses specifically adapted genetic structure to infect human population [12]. TB spreads through person-to-person contact from one infected person to others via airborne transmission of small droplet nuclei (0.5-5 μm) produced through coughs, sneezes, sing or even forceful speaking [13].

Further, these expelled small droplet nuclei undergo dehydration in the ambient environment and based on their particle size and aerodynamic properties one or more inhaled droplet nuclei deposit in the lungs [14]. It can affect practically all organs of the human body, but the lung is of particularly high incidence (80%) being designed as pulmonary or active TB. When it disseminates to extrapulmonary

regions it can affect any other part and is designated as extra-pulmonary or Latent TB.

Mycobacterium generally infects upper lobe of lungs by forming the Ghon focus [15]. Through inhalation, mycobacteria reach to the alveolar region of lungs where macrophages may phagocytose and kill the bacilli. After the initial interaction, the living bacilli enter and propagate within dendritic cells and alveolar macrophages (AM) [16], of the lungs at a rapid rate, signalling the production of IL-1-a, IL-1b, and other host pro-inflammatory cytokines. Complement, mannose, Fc, Toll-like receptor, surfactant protein A, CD14 and scavenger receptors present in macrophage surface are embroiled in the uptake of this mycobacterium. This is followed by interaction of mycobacteria with T lymphocytes forming differentiated macrophages into the histocytes and epithelioid which further amassed to form granulomas [17].

Further within granulomas, a variety of cells like differentiated macrophages, highly vacuolated macrophages and lipid-rich foamy macrophages, T cells, extracellular matrix components, and necrotic tissues are found [18]. Generally mycobacteria encircled by foamy macrophages within the granulomatous lesions are able to exist in a persistent or latent stage. At this stage, clinical symptoms will not be observed in the patient, but may show a positive response to a tuberculin skin test [19]. In this stage, MTb possess a lipid-rich cell wall which prevents the entry of antitubercular drugs (ATDs) and toxic host cell effectors molecules. In the persistent stage of life cycle, MTb also forms biofilms which are rich in free mycolic acids and provides additional resistance to ATDs penetration through it [20].

It is also reported that biofilms helps in the survival of the MTb in presence of anti-tubercular drugs in *in vitro* condition. Biofilms help in transmission of bacterial infection and offer protection to MTb from hostile environment which leads to decreased susceptibility to antibiotics [21]. In the granulomas, CD_4 cells T-Lymphocytes secretes cytokines which activates the macrophages to destroy the bacteria with which they are infected previously. Within macrophages, MTb is able to survive through hallmark mechanism of survival which includes suppression of intracellular Ca^{2+} concentrations by the *mycobacterium*, inhibition of reactive oxygen and nitrogen production, suppression of production of pro-inflammatory cytokines and chemokines, and prevention of phagosome maturation [22].

Symptoms

Active TB generally crops up in the lungs but can grip other organs also while secondary or passive TB lesions develop in peripheral lymph nodes, brain, lungs, larynx, liver, muscle, kidneys as well as in bones. TB can affect all body parts, but it rarely affects the heart, pancreas, skeletal muscles, and thyroid. The general signs and symptoms of the disease are a cough (>2-3 weeks), chest pain, bloody sputum, tissue destruction, fever, chills, fatigue, loss of weight and appetite, tiredness, severe headache, night sweats, briefness of breath [7]. Based on the status of TB in patients some differentiated symptoms can also be manifested.

In pulmonary disease is observed with a chronic productive cough, haemoptysis, enlarged lymph nodes, localized whereas constitutional symptoms including fever, loss of weight, or failure to thrive in children are associated with the production of pro-inflammatory cytokines. With the activation of T cell-mediated immunity, hypersensitivity phenomena are marked which includes condition like *phlyctenular conjunctivitis*, poncet's disease and *erythema nodosum*, whereas extrapulmonary disease is associated with lymphadenitis, meningitis, abdominal, bones and joints tuberculosis.

Current anti-tuberculosis chemotherapy

The objectives of current anti-tubercular chemotherapy is to treat patients without relapse with minimizing threat of loss of life and disability and to impede transmission of *MTb* to other persons along with avoidance of prevalence of drug resistance TB. Management of active TB by administrating a single drug should never be prescribed or be added to a failing regimen, as these lead to development of MDR-TB [23]. Thus multi-drug regimens over long periods of time are recommended for treatment of TB and drug resistant TB.

Intensive Phase – 2 months	**Under 50 kg**	**Over 50 kg**
RIF/INH/PZA/ETB Combination tablet 120/60/300/200 mg, 5 days per week	4 tablets/day	5 tablets/day
Continuation phase – 4 months	**Under 50 kg**	**Over 50 kg**
RIF/INH Combination tablet 150/100 mg Combination tablet 300/150 mg	3 tablets/day	2 tablets/day

Table 2: Dosage regimen used for treatment of new smear positive adult patients.

Combination of fATDs for minimum of 2 months period is recommended during initial intensive phase to reduce rapidly dividing bacilli load. Combination of 2/3 drugs are recommended for four months to sterilize lesions containing fewer and slow-growing bacilli, during the continuous phase. Recommended regimen for treatment of new smear positive adult patients are mentioned in Table 2 [24].

Details of five 'fATDs' (isoniazid (INH), pyrazinamide (PZA), ethambutol (ETB), rifampicin (RIF), and streptomycin (STR)) have summarized in Table 3. The 'second-line' ATDs used are ethionamide (ETD), kanamycin (KNM) or amikacin (AMK), capreomycin (CPM), vancomycin and para-aminosalicylic acid (PAS), etc.

Drug Name	BCS Class	Description	Mechanism of Action	Side Effect	T1/2	MIC	WS	Dose
Rifampicin	BCS-II	Broad spectra	Inhibits	Less appetite,	3.0-4.0	0.016-0.4		300

		antibiotic and the most active anti-TB drugs	bacterial RNA polymerase synthesis thus inhibits the nucleic acid synthesis	low urine excretion, Stomach upset, flu like symptoms, bleeding-Rashes and other hypersensitivity reactions				
Rifabutin		Used to prevent mycobacterium avium complex in people with HIV infection.	Inhibits bacterial RNA synthesis by binding strongly to the b-subunit of bacterial DNAdependent RNApolymerase	Itching, pale skin, easy bleeding, fever, chills, body aches, Flu symptoms, vision loss.	45	0.015 to 0.125		150
Pyrazinamide	BCS-III	As a pro-drug conversion. Inactive at neutral pH, but anti-TB at acid pH.	Inhibit fatty acid biosynthesis. Depletes and inhibit the membrane energy	Pains in joints and abdomen, Hepatitis, joint aches rashes and hypersensitivity reactions, gout	15 hrs	0.25–1.0		500
Ethambutol		As a drug target. Only affects *mycobacteria*	Inhibits arabinogalactan biosynthesis that leads to inhibit cell wall synthesis.	Reduced visual quality, Optic Neurititis	3-4	0.06–0.5		400
Isoniazid		As a pro-drug conversion. Antibacterial activity limited to mycobacteria	Inhibits the synthesis of mycolic acid thus interfering cell bacterial cell wall synthesis	Rash, Seizure, loss of memory, injury of lungs, Hepatitis, burning sensation in extremities, fatigue, fever	Fast acetylators: 0.5–1.6 h. Slow acetylators: 2–5 h	0.02–0.25		300
Rifapentine	NA	Treats only bacterial infections but not viral. Bacteriostatic agent	Inhibits DNAdependent bacterial RNA polymerase. Activate in Susceptible cells without inhibiting mammalian enzyme.	Hyperuricemia, pyuria, hematuria, urinary tract infection,	13	0.03-0.06		600

				proteinuria, neutropenia, anemia, and hypoglycemia				

Table 3: Details of first line antitubercular drugs.

Challenges for the present therapy

Contemporary TB therapy comprises a combination of ATDs administered by oral or parenteral route. It is worthy to note here that both of these conventional routes lead to sub- therapeutic levels of ATDs resulting in chances of occurrence of drug resistant TB owing to poor pulmonary distribution of drugs to lungs. ATDs may not penetrate into granulomas (protect MTb) in sufficient quantity, when administered with conventional formulation. Moreover prolonged treatments further worsen the condition as it increases the chances of occurrence of drug resistant strain, adverse effects, and insufficient drug distribution at the targeted organ.

Hence formulations delivering sufficient amount of drugs to the lungs and other targeted site is a promising avenue to explore and several researchers developed numerous such type of formulations such as liposomes, niosomes, microspheres, nanoparticles, nanoemulsions, nanosuspensions etc. These novel formulations offer numerous advantages like reduction in dose and dosing frequency (better patient compliance), targeting drugs to the macrophages (improve efficacy and reduce systemic toxicity), reduction in duration of therapy (offering more accumulation of drug at the targeted site), prevention of MDR-TB, stabilization of drugs and products.

Lipid based nano-formulations

In the era of nanotechnology, polymeric drug delivery systems are proposed as an alternative carrier for long term delivery of therapeutic agents along with their modified form. Yet, the number of polymeric micro and nanoparticles products in the market is still limited due to polymeric toxicity, solvent residues left after production, high cost of polymers, potentially toxic/allergic end products of polymers and the lack of feasibility for pilot plant scale-up method [25].

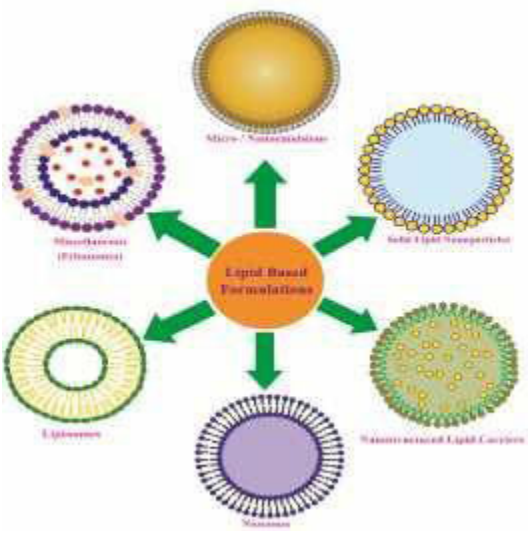

Figure 1: lipid based nano-formulations.

With the intention to prevail over these problems, lipid based drug delivery systems are proposed which gained a lot of attention and have taken the lead because of their inherited properties, biocompatibility and biodegradability, physiochemical diversity, lower toxicity, high incorporation efficiency for lipophilic drugs, improved bioavailability, stabilization of drugs, and controlled-release characteristics, and manufacturing at the pilot scale along with its suitability for drug delivery with different sites of administration [26]. Enormous attractive drug delivery systems which continuing to play in the versatile field and are coming under lipid based drug delivery systems are nano-emulsion, liposomes, niosomes, solid lipid nanoparticles, nano-structured lipid carriers, etc. Various types of lipid based nano-formulations are shown in Figure 1.

Nano or micro emulsions

Nano-emulsions an attractive kinetically stable liquid-in-liquid dispersion system, which formed spontaneously and comprises fine spherical oil in water dispersion covering size range of 10 and 100 nm. It promises to play a versatile role in diverse areas such as diagnostics, material synthesis, drug delivery, biotechnologies, food, cosmetics, and pharmaceuticals. The nano sized particle of nanoemulsion exhibited diverse properties like surface area per unit volume, tunable rheology, optically transparent appearance, robust stability. Since long it has retained its popularity in the field of ATDs delivery as the cells of phagocytic system and lipoprotein receptors of liver easily receive the drug following oral administration. Moreover these are thermodynamically stable and can be sterilized by filtration.

Various o/w nanoemulsion of RIF for i.v. administration was formulated by incorporating Sefsol® 218 (oil phase) and the aqueous

phase along with surfactant (Tweens®) and co-surfactant [27]. RIF incorporated microemulsion was found stable (validated by optical texture and phase separation) and changed into o/w emulsion at infinite dilution. The release of drugs was in a controlled manner as expected from o/w emulsion droplet [28]. More than 99% encapsulated homogeneous nanoemulsion showed initial burst effect (from 40 to 70% after 2 h) followed by a restrained release was noticed during *in vitro* drug release study. Optimized formulation of RIF was found stable and suitable for i.v. delivery. In a separate study physicochemical analysis of INH microemulsion was carried out and Non-Fickian release pattern of the nanoemulsion was established [29]. To observe changes in the microstructure of Tween 80-based microemulsion in the presence of ATDs viz. INH, PZA, and RIF separate investigation were expanded. The particle size ranged from 210 nm to 320 nm for these ATDs nanoemulsions. The formulations showed the controlled release with maximum drug release in 2h found to be 40%, 35%, and 10% for INH, PZA, and RIF, respectively [30].

Liposomes

Liposomes are gaining popularity as it offers benefits like selective passive drug targeting, better therapeutic efficacy, and flexibility to couple with site-specific ligands to achieve active targeting, increased drug stability, and reduced toxicity of encapsulating agents with improved pharmacokinetic effects. Liposomes are keenly taken up by macrophages and contents were released intracellular which immediately acts against MTb and hence it is considered as an emerging drug delivery system for the antimicrobial agents.

In animals infected with the Mycobacterium intracellular complex, liposome-encapsulated *streptomycin*, amikacin, gentamicin, and RIF exhibited greater efficacies than free drugs. INH and RIF loaded liposomes were developed to improve chemotherapy against MTb infected mice on the basis of detected CFUs, organomegaly, and histopathology. Liposome-encapsulated drugs at and below therapeutic concentrations were more effective than free drugs against tuberculosis. Elimination of mycobacterium from liver and spleen was found higher with liposomal drugs when compared with free drugs which offer more promising therapeutic approach for the chemotherapy of tuberculosis [31]. Clofazimine, Resorcinomycin A, and PD 117558 loaded liposome incorporated against several patients with microbial infection showed complete killing of bacteria at concentrations ranging from 8 to 31 μg/ml and thus liposomes, would help to reduce the toxicities of the drugs and could be used to target macrophages [32]. AMK encapsulated liposomes were found to be in high and sustained drug levels in infected tissues, exceeding the MIC for *M. avium* for at least 28 days while comparing with ciprofloxacin (CPF) encapsulated liposome in *M. avium* infected murine model. It clearly indicated that once-weekly and even once-monthly treatments with liposomal AMK could significantly reduce bacterial replication in infected tissues, extending the survival time of infected mice [33]. Numbers of CFU of *M. tuberculosis* in the spleen, liver, and lungs were determined one day after the last treatment of liposomal clofazimine (L-CLF) against MTb infection in acute, established, and chronic murine models.10-fold higher maximum tolerated dose of L-CLF demonstrated a dose response with significant CFU reduction in all tissues without any toxic effects. Bactericidal effect of L- CLF in the liver and spleen was validated due to absence of recurrence of *M. tuberculosis* growth. Thus L-CLF can be used as an effective therapeutic agent for the treatment of *M. tuberculosis* infections [34]. Gentamicin (GEN) encapsulated liposome was evaluated by assessing the efficacy of viable cell counts with beige mouse model of disseminated *M. avium* complex infection. Viable cell counts were determined from homogenized spleens, livers, and lungs. The results showed encapsulated GEN significantly decreased viable cell counts in the spleen and liver while comparing with the free GEN. In spleens and livers dose- related reductions in viable cell counts were noticed whereas none of the regimens claimed in sterilization of these organs [35]. Free and liposome encapsulated sparfloxacin were shown equal antimicrobial effect against Mycobacterium avium complex (MAC) in murine macrophage culture. However somewhat surprise results were found while both formulation administered *in vivo*. Liposome encapsulated sparfloxacin enhanced antibacterial effect against MAC infection *in vivo* due to its ability to localize in the mononuclear phagocyte (reticuloendothelial) system [36]. Surface modified stealth liposomes (tagging O-stearyl amylopectins) were found more stable in serum and accumulated more in lungs than in reticuloendothelial systems (RES) in normal and TB infected mice. Slow and controlled release of encapsulated contents from liposomes was observed from *in vivo* study. As compared to free drugs, INH and RIF encapsulated liposomes were found to be lesser toxic to peritoneal macrophages [37]. Natural killer activity of the macrophages and other phagocytosing cells were enhanced by tuftsin which led to enhance the nonspecific resistance of the host against parasitic and fungal infections. Intermittent treatments (twice weekly) with tetrapeptide tuftsin grafted rifampin liposomes were found to be 2000 times more effective than free drugs in lowering the lung bacilli load in infected mice. Thus homing of tuftsin grafted liposomes to macrophages may considerably improve the therapeutic efficacy of the liposomized rifampin [38]. Rifabutin (RFB) encapsulated liposomes exhibited lower bacterial loads in the spleen and liver while comparing with free RFB in an in TB-infected mice model. The levels of pathology in lungs were also found lower with encapsulated liposome treated mice. Prepared liposomes were localized in macrophages led to increase the efficacy of antibiotics against intracellular parasites avoiding long term use of the antibiotic in treatment of extra-pulmonary TB in human immunodeficiency virus co-infected patients [39]. In MTb infected mice treatment, biological evaluation was done by using swollen neutral PZA loaded liposomes (PZA-L). 10, 20 and 30 days after the last treatment dose of PZA-L, highly significant reduction in bacterial counts were noticed. From histopathological examination severity of infection in mice lungs were assessed. Infection in lungs treated with PZA-L was intermediate between drug free liposome and free PZA treated group of mice. It was found that amount of PZA-L equivalent to two-fifths the dose of daily free PZA exhibited superior antitubercular activity over free PZA in the management of experimental TB animal model. So PZA-L could be used to obtain effective targeting of the drug to subcellular organelles where the mycobacterial bacilli reside in infected macrophages [40]. AM were not activated by Spherical shaped levofloxacin encapsulated proliposome powders to produce cytokines and nitric oxide which were responsible for secondary inflammation. Superior antimycobacterial activity against *M. bovis*, *MTb* and intracellular *M. bovis* in macrophage cells were exhibited by developed proliposomes. Moreover it didn't show any toxicity in liver and kidney of Wistar rats signifying it's potential to combat TB [41,42]. Prepared rifapentine (RIP) loaded proliposome (RIPLP) were found with potent anti- TB activity, even though prepared by very high temperature spray drying method. Sensitivity of Tubercle bacteria to RIP proliposomal dry powder was studied through drug susceptibility testing and the sensitive concentration was found 10 μg/mL of RIP. From a Cytotoxicity study of A549 cells, it was also found safe in cells as

compared to pure drug whereas mortality was observed at higher doses. RIPLP treated animals showed dose-dependent lung toxicity.

Formulations	Lipid	Drug	Preparation Technique	Model	Comments	References
Liposomes	EPC	GEN	Modified plurilamellar vesicle	Beige Mice	Decreased CFU count observed both in spleen and liver in the infected mice. Dose related reduction in bacterial count.	[35]
	PG, CH, and PC	STR	N/A	Beige Mice	Improved antimicrobial activity against Mycobacterium avium complex	[43]
	DMPC And DMPG	CFM, RMP	Rotary evaporation	CultureSt rains of MAC 101	Showed highest killing effect.	[32]
	EPC	RMP	Sonication	Swiss albino mice	Super antitubercular activity	[38]
	EPC and CH	CPF	Solvent evaporation	Beige Mice	Improve therapeutic efficacy as compared to free drug	[36]
	PC, CH	INH, RIF	N/A	Mice	Less toxic to peritoneal macrophages. Sharp decrease in colony forming units in lungs, liver and spleen.	[31]
	EPC and CH	INH and RIF	N/A	Mice	Less toxic to peritoneal macrophages.	[37]
	SL	AMK and CPF	Modified ethanol injection method	Female C57BL/6 Mice	High and sustained drug level in infected tissues. Reduce bacterial count in the liver by more than 2 folds.	[33]
	DMPC and DMPG	CFM	Sonication	Mice	Showed significant CFU reduction in all tissues without any toxic effect	[34]
	PC, CH, DCP	INH, RIF	N/A	Murine	Reduce the mycobacterial load in lungs and other organs of infected mice.	[44]
	PC and CH	RIF	N/A	Wistar Albino rats	Improved delivery of rifampicin to macrophages. Reduce side effects. Drug targeting to lungs.	[45]
	DPPC, HPC and DSPC	CPM	N/A	N/A	Suitable for inhalable formulations. Enhanced drug accumulation at infected organ.	[46]
	LE and CH	RIF	Thin film hydration method	Wister rats	Liposomes were within respirable size range. Enhance of drug permeation in alveolar epithelium.	[40]
	LE and CH	RIF	Thin film hydration method	Wister rats	Liposomes were within respirable size range. Enhance of drug permeation in alveolar epithelium.	[39]
Lipidic Vesicles	DPPC and CH	INH, RIF, PZA, EMB	Thin film hydration method followed by Incubation	Wistar albino rats	High entrapment efficiency. Sustained/ prolonged drug release. Improved recovery/ uptake of drugs from target site/organ	[47]
Ligand- appended	SPC, PC, CH	RIF	N/A	N/A	Nontoxic to lung epithelial or alveolar macrophages. Do not activate inflammatory response in alveolar macrophages.	[48]
Proliposomes (Dry)	SPC, CH	INH	Spray Drying	N/A	Non-toxic to respiratory cells. Do not activate inflammatory mediators of alveolar macrophages.	[49]
Proliposomes	SPC, CH	LVF	Spray Drying	Rats	Enhanced activity against MTb. No renal and liver toxicity.	[41]

	HSPC, CH, STA or SA	RIP	Spray drying	Wistar Rats	Sustained drug release with longer retention of drug in lungs and highest targeting potential reduces systemic toxicity	[42]
	SPC, CH	PZA	Spray Drying	Calu-3, A549 and NR8383	Less toxic. No production of inflammatory mediators. No Renal and liver toxicity.	[50]

Note: Lipids:-SA: Stearic Acid; PC: Phosphatidyl Choline; SL: Soya Lecithin; MCT: Medium Chain Triglyceride; PA: Palmitic Acid; TS: Tristearin; PR80: Phospholipon R80H; COMP: Compritol® 888 ATO; GMS: Glycerylmonostearate; GDB: Glyceryldibehenate; GTS: Glyceryltristearate; CP: Cetylpalmitate; MYG: Mygliol 812; PRE: Precirol® ATO 5; **Drugs:-** RIF: Rifampicin; INH: Isoniazid; PZA: Pyrazinamide; EMB: Ethambutol; STR: Streptomycin; RIB: Rifabutin; CPF: Ciprofloxacin; RIP: Rifampin; MIC: Minimum Inhibitory Concentration.

Table 4: Outcomes of liposomes and pro-liposomal based formulations in the management of TB.

It increased the cytotoxic threshold (IC_{50}) as compared to pure RIP in A549 cell lines validating its potential for the treatment of pulmonary TB [42]. Apart from above, some additional liposomal formulations along with their outcomes in the treatment of TB are summarized in Table 4.

Niosomes

Niosomes signify an emerging class of novel vesicular systems, which are structurally very much similar with the liposome, derived from nonionic surfactants (monoalkyl or dialkyl polyoxyethylene ether) and charged phospholipids (stearyl amine and diacetyl phosphate). Latter is obtained from cholesterol hydration [51]. Niosomes are superior lipid based carrier over liposomes owing to their better chemical stability, greater osmotic activeness, easier pilot plant scale-up feasibility, cheaper cost of production along with longer storage time. Beside it can overcome the drawbacks associated with sterilization and offers the stability of phospholipidic components of liposomes upon light exposure even at room temperature. Niosomes can accommodate hydrophilic drugs inside its core whereas lipophilic drugs are encapsulated in hydrophobic provinces at the same time.

Considerably higher drug concentrations were found in the targeted organs via intraperitoneal (i.p.) route of administration in contrast to i.v. route. A significant increase in accumulation of the RIF-loaded niosomes was observed in the lungs after intra-tracheal administration. With RIF-N particle, 90% release of RIF in 48hrs was achieved during the *in vivo* study. Higher (65%) localization of drug was found following administration of RIF-N as compared to free RIF (15%) [52], and the bio-distribution of niosomes with smaller sizes consisting of different sorbitan esters and cholesterol [53]. Sustained drug release with higher cellular uptake was achieved with niosomes at the treated site resulting in reduction in the drug dose, toxicity and dosing frequency which led to improved patient compliance for effective treatment of TB [54]. Particle size, entrapment efficiency of INH incorporated niosomes were found 2.3 μm and 80% respectively. 90% of the drug released in 48 hrs was found from *in vitro* study. Niosomal formulations offered higher accumulation of the drug in visceral organs (lung, kidney, liver, spleen) resulting in fewer incidences of toxicity of than free drug [55]. Ethambutol loaded niosomal formulation offered controlled drug release with higher drug targeting effect to mice lungs for a prolonged period of time. Decreased root specific lung weights along with decreased bacterial counts were observed from lung homogenates. Thus offered higher efficacy and safety compared with the free drug [56].

The highly stable innocuous RIF and INH niosomes with higher encapsulation efficiency were developed which offered to solve the problem of MDR in the case of tuberculosis. Fickian or diffusional release had been observed for RIF and INH and a non-Fickian release mechanism for PZA from *in vitro* study [57]. Outcomes of various niosomal drug delivery systems in the treatment of TB are summarized in Table 5.

Solid lipid microparticles

RIF loaded lipid microsphere (R-LM) delivered the drug through intranasal route to alveolar macrophages for achieving improved therapeutic efficacy in tuberculosis (TB) and TB/HIV patients with reduced hepatotoxicity. *In vitro* uptake of unencapsulated R-LM by alveolar macrophages was over 4 times more than that of unencapsulated RIF, whereas the *in vivo* uptake was 30 times more. R-LM could deliver the encapsulated drug effectively to alveolar macrophages *in vitro* and *in vivo* was confirmed through Flow cytometric analysis and confocal laser scanning microscopy. Intranasal administration of R-LM to normal mice resulted in preferential pulmonary uptake of the drug and lower levels in the blood and liver compared with administration of free RIF [58]. RIF loaded lipid microspheres (R-LMs) preferred with an intention to improve therapeutic efficacy in immunocompetent hosts by decreasing RIF - nevirapine interaction and enhancing bacteriostatic effect against MTb in immunodeficient hosts. R-LMs were administered through intranasal route which showed a significant bacteriostatic effect against MTb-H37Rv. In immunodeficient BALB/c nude mice, the efficacies of R- LMs in lungs were found higher whereas in immunocompetent BALB/c mice and found unchanged while comparing with the results of oral RIF groups. Cmax and AUC0–3 of nevirapine were decreased from 32.2% to 11.9% and 30.5% to 12.4% respectively with the administration of R-LMs when compared to oral RIF. Thus R-LM may possibly improve antitubercular activity in the immunodeficient host and minimize drug interaction between RIF and nevirapine [59]. Cytotoxicity and cell internalization ability of RIF loaded solid lipid microparticle (R-SLMs) were evaluated on murine macrophages J774 cell lines by MTT test, cytofluorimetry, and confocal laser microscopy. SLMs exhibited aerodynamic diameter (fit for transportation up to the alveolar region), negatively charged surface (promote uptake by the macrophages and preserved drug antimicrobial activity). The negligible *in vitro* release of RIF indicated the capacity of the SLMs to entrap the drug preventing its spreading over the lungs fluid. *In vitro* studies on J774 cell lines reported that SLMs as non-cytotoxic and ability to be taken up by cell cytoplasm. The SLMs, showed features suitable for the pulmonary delivery and for inducing endocytosis by alveolar macrophages, for this reason, it may possibly be considered as a promising efficacious TB therapy using a Dry Powder Inhaler device [60]. Smaller particle size with improved sustained release properties

was observed with RIF encapsulated microparticles. Stable formulations exhibited comparatively lower MIC range against various pathogenic microorganisms resulting in reduction in a dosing frequency and would be helpful for TB treatment [61]. Outcomes of various solid lipid microparticle based drug delivery systems in the treatment of TB are summarized in Table 5.

Formulation	Lipid	Drug	Preparation technique	Model	Comments	References
Lipid Microsphere	SPC and SO	RIF	Modified Homogenizati on	Male Wistar rat	Improved drug uptake with reduced hepato-toxicity, Better stability. Suitable for TB/HIV patients.	[50]
	SPC and SO	RIF	Homogenizati on	Immunoco mpetent and Immunodefi cient Mice	Improved anti-tubercular activity in immune deficient host. Reduces RIF- Nevirapine interaction.	[51]
	SA, STH	RIF	Sonication	Murine macrophage J774 cell lines	Suitable for the inhalation. Induce endocytosis by alveolar macrophages	[52]
Solid Lipid Microparticle	MO and P90G	RIF	Melt homogenization technique	Male Wistar Rat	Stable against gastric acid degradation. Reduce the frequency of dose administration. Increase bioavailability and Reduce the side effects.	[53]
Niosome	CH, triton- X	RIF, INH	N/A	Wistar rats	Effective targeting of the RIF to the lymphatic regions.	[45]
	SMS, CH, DCP	INH	Reverse phase evaporation method	J744 A.1 mouse macrophage cells	Optimum level of drug entrapment efficiency. Reduced dose as well as dosing frequency and Toxicity.	[46]
	CH, DCP, STA,	PZA	Vortex Dispersion Method	Guinea Pig infected with H37Rv strain.	High drug entrapment efficacy	[62]
	Triton X 100	RIF, INH	N/A	N/A	High drug entrapment efficiency for both RIF and INH niosome.	[49]
	CH, DCP, STA	EM B	Thin-film hydration	Swiss Albino mice infected with H37	Increased drug-loading efficiency. Zeta potential up to neutral values. Increased drug targeting efficacy on lungs	[48]
Microemulsion	OA	RIF	N/A	N/A	Stable RIFmicroemulsion. Conversion of microemulsion into o/w emulsion at infinite dilution.	[28]
	OA	INH	Self- Emulsification	N/A	INH microemulsion was found stable. Release of INH was sustained/ controlled manner	[29]
	OA	RIF, INH, PZA	N/A	N/A	Drugs in single and mixed drug formulations follow non-Fickian release behaviour except for RIF in in pH 7.4 release medium	[30]
Nano- emulsion	Sefsol	RIF	Aqueous phase titration method	N/A	Stability of oil-in-water (o/w) formulation more than 19 months.	[27]
Aerosol particle	DPG PC	PAS	Spray Drying	Rat	Rapid systemic drug onset and high concentration in the lung lining fluid in animal model	[63]
Dry powder lipopoly	SA	RIF	Spray Drying	THP-1 cell line	Colocalization of rhodamine- entrapped nanomicelles for targeting cell compartments of alveolar macrophages.	[64]
Supergenerics Inhalable Powders	Oleat e,lino leatea nd linole nate	CP M	Spray Drying	Chicken chorioallant oic membrane assay	CPM oleate and linoleate have equal efficacy against *M. tuberculosis* but oleate form shows lowest toxicity	[65]

Abbreviations:- Lipids:-CH-Cholesterol; DCP-Dicetyl phosphate; DPGPC-1,2-dipalmitoyl-snglycero-3- phosphocholine; MO-Moringa Oil; OA-Oleic Acid; P90G-Phospholipon 90G; SPC-Soya phosphatidylcholine; SO- Soybean oil; SA-Stearic acid; STA-Stearylamine; SMS-Sorbitanmonostearate; STH-Sodium taurocholate hydrate. **Drugs:-**RIF-Rifampicin; PZA-Pyrazinamide; EMB- Ethambutol; INH- Isoniazid; AMK-Amikacin; CPM- Capreomycin; PAS- Para amino salicylic acid.

Table 5: Outcomes of lipid microspheric, microparticle, niosomal, emulsion and miscellaneous lipid based formulations in the management of TB.

Solid lipid nanoparticles

Solid Lipid Nanoparticles (SLNs) are made up of solid physiological lipids generally dispersed in water or an aqueous surfactant solution. The matrix of SLNs consists of closely packed perfect crystalline solid lipid leaving very few empty spaces resulting in poor drug loading, moreover on storage due change in packaging of lipids expulsion of drug content takes place. Simultaneously it has had plenty of unique characteristics which are good tolerability (as physiological lipids), free from organic solvent, pilot plant scale-up feasibility, control and/or target drug release and have the capacity to incorporate both lipophilic and hydrophilic drugs, and improved drug stability [25]. Chemotherapeutic potential of nebulized ATDs (RIF, INH, and PZA) loaded solid lipid particles (SLPs) were evaluated against experimental guinea pig tubercular model. Developed SLPs possessed an appropriate mass median aerodynamic diameter apposite for bronchoalveolar drug delivery. The nebulized SLPs could be detected in the plasma from 6 h onwards up to 120 h whereas free drugs could not be detected in the plasma beyond 12 h following their iv/oral/aerosol administration. Moreover, with a single nebulization, a sustained drug release for 7 days and 5 days were attained in the organs (liver, lungs, and spleen) and plasma respectively. Therapeutic drug concentrations in plasma, mononuclear phagocyte riched organs (lungs, liver, and spleen) were achieved up to 8 and 10 days respectively with a single oral administration of SLP formulation when compared with the free drug which cleared within 2 days.

It has been found that the *MTb* H37Rv infected guinea pig, 5 oral doses of drug loaded SLPs, in every 10th day was therapeutically equivalent to the 46 daily oral doses of free drugs. Thus nebulization of SLP-based ATDs improves bioavailability with reduction in dosing frequency offering better patient compliances [66]. First line ATDs viz. INH, PZA, RIF, EMB loaded SLNs showed a significant improvement in relative bioavailability in the plasma (6 times) and brain (4 times) with respect to the free drug solution at the same dose [67]. 8 fold increases in bioavailability of RIF were achieved with administration of R-SLNs when compared with free drug [68]. R-SLN also evades INH induced degradation, *in vivo* conditions, when administered concomitantly with free INH to rats. Further a highly hydrophilic molecule, STR was encapsulated in SLNs with entrapment efficiency of more than 60% [69]. A 5-fold increase in plasma bioavailability was achieved with administration of EMB loaded SLNs as compared to free drug solution. Therapeutic drug concentrations of RIF, INH, and PZA loaded SLNs were maintained in the plasma and in the organs (lungs, liver, and spleen) for 8 days and10 days respectively whereas free drugs were cleared by 1–2 days after a single oral administration of SLNs to mice. 5 oral doses of SLNs had equivalent therapeutic benefits with 46 daily doses of oral- free drug observed in MTb H37Rv infected mice [70]. INH loaded SLNs showed improved bioavailability and prolonged effect, minimizing associated side effects at peak plasma concentrations. Optimized SLNs formulation was expected to bypass reticuloendothelial system prolonging circulation time of the drug. Pharmacokinetic studies observed significant improvement in relative bioavailability in plasma (6 times) and brain (4 times) while comparing with free drug solution. Insignificant changes in liver concentration were found along with slow release of INH indicating low incidence of hepatotoxicity [71]. Pharmacokinetic studies of RIF loaded SLNs (R-SLNs) following Single oral dose (50 mg/kg) using Wistar rats indicated 8.14 times higher plasma bioavailability with sustained levels for 5 days while comparing with free RIF. Pharmacodynamic parameters viz. TMIC (time for which plasma levels were above MIC), AUC0-∞ /MIC and Cmax/MIC for R-SLNs were greater than free RIF by 2.5, 8.2 and 6.6 times, respectively. Improved pharmacokinetic profile of R-SLNs offered reduction in dose and dosing frequency, resulting in lesser or no hepatotoxicity [72]. Prepared RFB loaded SLNs were able to endure harsh temperature condition (confirmed by dynamic light scattering) and complete release of drug was noticed from release study. *In vitro* cell line studies with THP-1 cells differentiated in macrophages showing a nanoparticle uptake of 46% and 26% for glyceryl di-behenate and glyceryl tristearate SLNs, respectively. Low cytotoxicity was observed with SLNs from Cell viability, proposing SLNs as new potential vehicles for pulmonary delivery of ATDs [61]. Wheat germ agglutinin (WGA) conjugated RIF loaded SLNs (WRSLNs) were prepared and Conjugation efficiency was determined using fluorescent spectroscopy and Bradford assay. Even after coupled with the nanoparticles, bio-recognition activity and sugar-binding specificity of WGA was retained as validated from haemagglutination test. Interaction of WRSLNs with porcine mucin was found during *in vitro* experiment when compared with the non-conjugated nanoparticles. WRSLNs were stable in the presence of electrolytes up to 1.0 M concentration. Prepared WRSLNs showed narrow size distribution, controlled drug release, retention of biorecognition activity and good physical stability against electrolyte induced flocculation [62]. RIF and INH both undergoes degradation in gastric pH up to extent of 26.5% and 1.43% respectively. The degradation of RIF further enhanced (48.8%) with co-presence of INH due to their interaction. RIF and INH loaded SLNs which were able to prevent their degradation from acidic gastric pH and drug-drug interactions. The RIF-SLNs were able to bring down its extent of degradation up to 9% when present alone whereas co-presence of both INH and RIF-SLNs, degradation of RIF dropped down to 20% from 48.8%. But co- administration of (RIF-SLNs + INH-SLNs) reduced the degradation up to 12.35%. Prepared formulations were able to enhance bioavailability, reduce dose with lesser side effects, and to target specific site in the body like brain in case of cerebral tuberculosis. The study indicated that SLNs can limit their interaction so that the risk of failure of therapy can be overcome [63]. RFB loaded mannosylated SLNs evaluated for their toxicity, targeting potential, alveolar macrophage uptake, hematological studies, and *in vivo* studies. It was noticed from *ex vivo* cellular uptake study that there was a six-fold increase in drug uptake by the alveolar macrophages owing to mannose coating. Mannose-conjugated systems were observed to be less immunogenic from hematological studies and hence apposite for sustained delivery. The manosylated SLNs may possibly employed for an effective and targeted delivery of RFB with reduced side effects [64]. For prolonged ciprofloxacin release in a controlled manner, SLNs were prepared having entrapment efficiency 38.7% and zeta potential value -28 mV.

Ciprofloxacin loaded SLNs showed sustained drug release (Higuchi Model) avoiding "burst effect" of the free drugs for up to 80 h and which could act as promising carriers in infective conditions [65]. Prepared rifampin loaded SLNs showed sustained release of drugs for 72 hrs with strong antimycobacterial efficacy (MIC eight-time lesser than free drug) against *Mycobacterium fortuitum* in *in vitro* condition [66]. MTT (3- [4,5-dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide) assays were employed to examine the cytotoxicity of RIF loaded SLNs (RIF-SLNs) in alveolar macrophages (AMs) and alveolar epithelial type-II cells (AECs) and the viability of AMs and AEC were found above 80% signifying low toxicity to both AMs and AECs. Higher amount of RIF was found in AMs each time observed during *in vivo* study signifying selective delivery of drugs to specifically to AMs

[67]. Outcomes of various SLNs based drug delivery systems in the treatment of TB are summarized in Table 6.

Formulations	Lipid	Drug	Preparation Technique	Model	Comments	References
Solid Lipid Nanoparticle	SA	RIF, INH, and PZA	Emulsion solvent diffusion	MTb H37Rv infected Mice	Therapeutic drug concentration maintains in the plasma for 8 days to 10 days. Reduces dosing frequency.	[58]
	SA	RIF, INH, PZA	Emulsion solvent diffusion	MTb H37Rv infected guinea pigs	Sustained release up to 5 days. Reduce dosing frequency. No evidence of any biochemical hepato toxicity.	[54]
	SA and PC	CPF	Warm o/w microemulsion	N/A	Promising formulations for prolonged release of drug for local delivery.	[65]
	TS And SL	RIB	Modified solvent injection method	N/A	Manosylated SLNs are better suited for site specific drug targeting.	[64]
	CP	RIP	Modified micro- emulsion method	*In vitro*	MIC of SLNs was found to be eight times lesser.	[66]
	CO MP	RIF, INH, STR, EMB	Hot or cold high pressure homogenization	Rat	Improved bioavailability, protection against INH induced degradation	[55-57]
	CO MP	RIF and INH	Modified Microemulsificat ion	N/A	Enhance bioavailability. Site specific drug targeting (Brain).	[63]
	SL, SA, PA	RIF	Modified lipid film hydration method	Sprague– Dawley rats	Increase in drug content in alveolar macrophages with targetted drug delivery.	[67]
	CO MP, SA	INH	Micro emulsification	Rat	Higher drug loading with slow release of drug. Low incidence of hepatotoxicity. Bye passes fast pass metabolism.	[59]
	CO MP	RIF	Microemulsificat ion	Wistar Rat	Improved pharmacokinetic profile, reduced dose and dosage frequency, no hepato-toxicity	[60]
	GMS, SA	RIF	Emulsification – solvent evaporation	N/A	Controlled release of drug, retention of biorecognitive activity with good physical stability.	[62]
	GDB and GTS	RIB	Hot high shear homogenization	A549, Calu-3 , ATCC1 TIB-202TM Cell line	Low Cytotoxicity, Better Physical Stability, Improved release profile	[61]
Nanostructured Lipid Carrier	MCT	RIF	Film homogenization	Cell LIne NR8383	Cationic mannosylated NLCs showed higher drug uptake capacity. Showed superior lung targeting effect	[68]
	PRE	RIB	High-shear homogenization and Ultrasonication	A549, Calu-3, and Raw 264.7 cells	Mannose coated NLCs improve cellular uptake with site specific drug targetting. Fairly shelf life stability strategy.	[69]
	CP and MY G	RIF and RIB	Ultra-sonication	RAW, Calu-3 and A549 cell lines	Manosylated drugs showed better drug uptake capacity and better drug targeting anti-tubercular effect.	[70]

Note: Lipids:-SA: Stearic Acid; PC: Phosphatidyl choline; SL: Soya lecithin; MCT: Medium chain triglyceride; PA: Palmitic acid; TS: Tristearin; PR80: Phospholipon R80H; COMP: Compritol® 888 ATO; GMS: Glycerylmonostearate; GDB: Glyceryldibehenate; GTS: Glyceryltristearate; CP: Cetylpalmitate; MYG: Mygliol 812; PRE: Precirol® ATO 5; **Drugs:-** RIF: Rifampicin; INH: Isoniazid; PZA: Pyrazinamide; EMB: Ethambutol; STR: Streptomycin; RIB: Rifabutin; CPF: Ciprofloxacin; RIP: Rifampin; MIC: Minimum Inhibitory Concentration

Table 6: Outcomes of solid lipid nanoparticles and nano-structured lipid carrier based formulations in the management of TB.

Nanostructured lipid carriers (NLCs)

NLCs, the second generation of colloidal lipid nanoparticles which gained huge attention over the past few years as a promising drug carrier overwhelming most of the drawbacks associated with SLNs. The presence of liquid lipids with different fatty acid C- chains produces NLCS with less organized crystalline structure and thus providing better loading capacity for drug accommodation. Liquid lipids are better solubilizers of drugs than solid lipids. These above characteristics proved its specialty as it retain all the advantages of SLNs in one hand and at another hand, it simultaneously overcomes most of their limitations such as poor long term stability, low drug loading capacity, and a possibility of drug expulsion, pilot scale production. Due to its unique characteristics, several attempts have been taken by the researchers to utilize NLCs as committed nanocarriers for successful delivery of anti-tubercular drugs.

The presence of octadecyl amine in RIF loaded cationic mannosylated NLCs was responsible for achieving higher drug loading capacity due to its cationic properties. Higher drug encapsulation was possible due to the interaction of as ammonium group of Octadecylamine with the phenolic hydroxyl group of RIF via an ionic bond. The cationic property was helpful in improving the distribution of drugs in the lungs. Modification of NLCs was done by inserting Mannosylated cholesterol to the NLCs through cholesterol residue which is responsible for cell specific targeting action. Optimized Mannosylated RIF NLCs formulation was found with minimum cytotoxicity which would suitable for systemic administration of drugs when compared with RIF NLCs and RIF suspension [68]. RFB loaded mannosylated NLCs delivered to alveolar macrophages for improvement of therapeutic index. The diameter of the produced particles was found to be around 200 nm appropriate for lung deposition with passive targeting, validating the proposed pulmonary route of administration. RFB loaded NLCs were efficiently internalized by the alveolar macrophages and was able to release bactericidal concentrations of the drug (>100 and <1,000 μg ml^{-1}). Sugar receptors present in the mannosylated RFB-NLCs offered to improve the cellular uptake of drug with active targeting strategy. Cytotoxicity free NLCs were observed with high storage stability with pH-sensitive drug release and drug release was faster at acidic pH found in phagosomes (pH~6.2) and phagolysosomes (pH~5.0) as compared to neutral pH. These outcomes pose a strong logic that the developed nanocarrier can be used as a potential carrier for safer and more efficient management of tuberculosis by exploiting the pulmonary route of administration [69]. Mannose coated and uncoated RIF and RFB loaded NLCs individually by ultrasonication method. Mannosylated NLCs showed high Zeta potential, Polydisperse particles were observed but within breathable size range suitable for pulmonary administration and subsequent deposition in lung. Formulations had passive targeting strategy which could increase treatment efficacy, with better patient compliance. High Zeta potential value for all formulations suggested fairly good shelf life stability, moreover, positive zeta potential observed in mannosylated NLCs (MNLCs) signifying successful completion of mannose coating. It was also reported that mannosylation method had no influence on particle shape, drug loading. Mannose surface modification was done to take advantage of sugar receptors available in alveolar macrophages to improve cellular uptake with an active targeting strategy and these objectives were achieved as in this study it was observed that MNLCs had higher drug uptake efficiency in model cells and alveolar macrophages along with cell-specific targeting when compared with simple NLCs. Cytotoxicity of the MNLCs was studied by MTT assay and lactate dehydrogenase assay and was ascertained that formulation was safe and suitable for pulmonary administration with targeted action by the investigator [70]. Outcomes of various NLCs based drug delivery systems in the treatment of TB are summarized in Table 5.

Miscellaneous lipid based formulations

The RIF loaded amphiphilic lipopolymeric nanomicelles were formulated with inhalable size particle which helped in absorption into the cell through proton sponging effect. Non-endocytic entry of lipopolymer into the SABPEI 5050 with larger colocalization of GFP-tagged M. smeg with rhodamine entrapped nanomicelles was observed inside the THP-1 differentiated cell and thus could be employed for targeting *Mycobacterium tuberculosis* residing inside the phagosome of alveolar macrophage [71]. Para-aminosalicylic acid (PAS) loaded large porous lipid particles for direct delivery into the lungs via inhalation showed better deposition of the drug throughout the respiratory tract. Upon insufflations in rats, the concentration of the lung lining fluid and tissue concentration were 148 μg/ml and 65 μg/ml at 15 min respectively. Therapeutic concentrations of PAS in the lung tissue were found even after 3 hrs of complete clearance in the lung lining fluid and plasma. Thus it could reduce total dose delivery and be suitable for administration of RIF, aminoglycosides or fluoroquinolones [72]. The morphology and particle sizes of capreomycin inhalable lipid powders were are fitted for inhalation. Efficacy against MTb exhibited by capreomycin oleate and linoleate were same as that of capreomycin however it was more than that of capreomycin linoleate. Results of *in vivo* toxicity studies showed that capreomycin oleate exhibited its lowest toxic potential suggesting it be used as super generics in pulmonary tuberculosis treatment [73]. Outcomes of various miscellaneous lipid based drug delivery systems in the treatment of TB are summarized in Table 6 [74-86].

Conclusion

Though the contemporary therapy for TB is effectual, and it is narrated with severe disagreeable side effects leading to noncompliance prescribed regimens, most of the polymeric drug delivery systems are not universally acceptable owing to their numerous limitations. In this status lipid based nano-formulation based drug delivery systems are emerged as a promising drug carrier to substitute most of the polymer-based formulations because of it is going to counterbalance polymer and drug associated limitations. Further blending, the principle of nanotechnology with lipid systems introduced lipid nano-carriers, which are considered to be the latest

generation of lipid carrier having an enormous chemotherapeutic potential.

Research is still needed to better understand the absorption enhancing mechanisms of lipids; to find better predictive tools for assessing the *in vivo* behaviour of various lipids with different types of drug molecules; development of regulatory guidelines for characterization of lipid based formulations; and techniques for enhancing the stability of lipid based systems. The study suggested that lipid based antitubercular drug could offer an economical chemotherapy with reduced dosing frequency and improved patient compliance for better management of tuberculosis. The real success of the lipid system may be attained with the development of cost effective, bio-available, nontoxic, and stable formulation to address the limitation of anti-TB chemotherapy making the therapy affordable to the last person in the queue with need.

Conflicts of Interest

The authors have no conflicts of interest.

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