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Urgency of novel anti-tuberculosis strategies: a prospective challenge

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Abstract

Tuberculosis (TB) is a fatal infectious disease, caused by bacterium called *Mycobacterium tuberculosis,* killing nearly two million people every year. The increasing incidence of resistance of *Mycobacterium tuberculosis* strains to currently used drugs due to inadequate dosing and incomplete treatment regimens is a major factor contributing to the current TB epidemic. Due to this concern, scientists have renovated their approaches to the finding of novel anti-tuberculous drugs and the development of the nanoparticle-based delivery system to subdue technological drawbacks and improve the effectiveness of therapeutic drugs. This article deals with the following areas: first, the present status of the development of new anti-tuberculous drugs is reviewed. This includes the newly approved drugs bedaquiline and delaminid, and other new promising anti-tuberculous agents, such asnitroimidazoles, diarylquinolines and oxazolidinones; and second, the development of new nanotechnology-based therapies which can be used for the treatment of TB is reviewed. This includes liposomes-based, niosomes-based and microemulsions-based anti-tubercular drug delivery strategies.

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Introduction

Tuberculosis (TB), an infectious disease caused by acid fast pathogenic bacteria *Mycobacterium tuberculosis* (*Mtb*), is a major public health concern with over 2 billion people currently infected, 8.6 million new cases per year, and more than 1.3 million deaths annually (Baldwin *et al.*, 2015).Major factors that are sustaining the current TB epidemic include expanding human immunodeficiency virus (HIV) infection and its association with active TB disease and increasing resistance of *Mycobacterium tuberculosis* strains to the most-effective (first-line) anti-TB drugs. Although drug-sensitive (DS)-TB is curable with a 6–9-month regimen comprising the four first-line drugs, isoniazid, rifampicin, ethambutol, and pyrazinamide, the emergence and spread of multi- and extensively drug-resistant (MDR and XDR) strains of *Mtb* has greatly complicated the control of TB. In 2014, an estimated 480,000 people worldwide developed MDR-TB and 190,000 died from this form of the disease. In addition, in 2015, cases of XDR-TB were reported in 105 countries, and an estimated 9.7% of people diagnosed with MDR-TB developed XDR-TB. As a consequence of the inexorable rise in drug resistance over time, reports of "totally" drug-resistant (TDR)-TB, resistant to all first- and second-line antitubercular drugs, have now become increasingly common (Parida *et al.*, 2015).

Current treatment regimens

The current drug regimen combination consists of four first line drugs- isoniazid (INH), rifampicin (RFM), pyrazinamide (PZ) and ethambutol (ETB). This regime can be used for the effective treatment of sensitive, non resistant strains of *M. tuberculosis*. There are two phases of drug administration. Initially during the "intensive" phase of the first two months of the treatment, all the above four drugs are administered on alternate days followed by the "continuous phase" of next 4-6 months in which only two drugs, rifampicin and isoniazid are administered to eradicate the remaining *bacilli* that have entered a dormant, slowly replicating latent phase (Luciani *et al.*, 2009; Udwadia *et al.*, 2012). The above four drugs are effective for the active growers populations of bacteria but no drug is effective for dormant bacilli (Dover *et al.*, 2011).

Poor patient complaisance

The above drug regime has many flaws which have led to the poor patient complaisance. The patient has to face severe side effects such as nausea and hepatotoxicity. For this reason the patients do not remain stick to the regime. Only a highly disciplined patient can stick to the regime till the end of the treatment. To reduce the number of TB deaths and infections WHO has launched DOTS (Directly observed treatment short course) strategy that consists of five key elements: government commitment, diagnosis through bacteriology, standardized and supervised treatment, uninterrupted drug supply and regular program monitoring (Raviglione *et al.*, 2006; Sharma *et al.*, 2006). The poor patient compliance is a major factor which results in the development of resistance to first line drugs (Munro *et al.*, 2007).

Latent form and Relapse of TB

All the TB drugs are effective as long as the *M. tuberculosis* remains in the multiplying phase. Soon after the multiplying phase, the bacteria enters a persistent phase in which it stops multiplying and undergoes hibernation. Rifampicin is the only drug which is effective against the persistent *M. tuberculosis* but has weak activity which elongates the duration of the treatment for several months. After a few months of cessation of the drug combination treatment, there are chances of relapse of the persistent form into active form. The disease may even develop in more nasty form i.e., drug-resistant tuberculosis (DR-TB) (Keshavjee *et al.*, 2012).

Drug-resistant Tuberculosis (DR-TB)

Although this treatment has a high success rate, the utility of this regimen is limited by compliance issues, which has resulted in the rise of strains that are resistant to some or all of the first- and second-line antibiotics (Loddenkemper *et al.*, 2010). These strains, called multidrug resistant (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR)

strains of Mycobacterium tuberculosis (Sloan *et al*., 2013).Multidrug-resistant tuberculosis (MDR-TB) is the disease in which the strains of *M. tuberculosis* become non responsive to the two most effective first line drugs. Patients with extensively drug-resistant tuberculosis (XDR-TB) are resistant to rifampicin, isoniazid, and fluoroquinolones and at least one injectable antibiotic (Esposito *et al*., 2014). Total drug resistant tuberculosis (TDR-TB) is a condition in which the patients become resistant to all the first and second line drugs ultimately leading to death (Arya *et al*., 2014).

First Line Anti-TB drugs

Mechanism of Action and Resistance

A better understanding of drug resistance mechanisms in *M. tuberculosis* is crucial for the development of rapid methods for drug resistance detection and new anti-TB drugs to treat MDR-TB/XDR-TB patients. Most Mtb resistance observed in the clinic can be attributed to independent, spontaneous mutations that interfere with the drug binding to the target protein, reduce pro drug-activating enzymes, or over express an essential target (Cohen *et al*., 2014). For a more detailed analysis of the molecular basis of resistance to currently used agents, we refer readers to a review by Palomino *et al.* (2014). Current first line antitubercular drugs are commonly associated with specific resistance mutations (Table 1) (Hoagland *et al.,* 2016).

Table 1. First line antitubercular drugs and their related resistance mechanisms.

Drug	Mechanism of action	Common mechanism of Resistance	Source
Rifampicin	RNA synthesis inhibition	Mutation of rpoB induces a conformational change at β-subunit of RNA polymerase causing a decrease in binding affinity	Talenti *et al*., 1993 Ramaswamy *et al*., 1998
Isoniazid	Mycolic acid biosynthesis inhibitor and effects on DNA, lipid, carbohydrate, and NAD metabolism	KatG suppression causing decreased prodrug activation, and a mutation in the promoter region of InhA causing an over expression of InhA	Zhang *et al*., 1992 Vilcheze *et al*., 2006
Pyrazinamide	Not fully resolved, may include membrane potential disruption	Mutations in pncA reducing conversion to active acid form	Scorpio *et al*., 1996 Sekiguchi *et al*., 2007
Ethambutol	Arabinogalactan biosynthesis inhibition	Mutations in embB at codon embB306	Safi *et al*., 2008

Second Line Anti-TB Drugs

Mechanism of Action and Resistance

The second line anti-TB drugs are only practiced to treat TB that is resistant to first line therapy (i.e., for extensively drug-resistant tuberculosis (XDR-TB) or multidrug-resistant tuberculosis (MDR-TB).

Unfortunately these drugs induce serious side effects according to WHO Report 2010. Specific resistance mutations (Table 2) are found to be associated with current second line antitubercular drugs (Hoagland *et al.,* 2016).

Development of novel treatment regimens

Several antitubercular drugs have been in the use for five to six decades. Second line drugs can be administered but they are very expensive and their duration of treatment is about 2 long years. These drugs are required to be administered in very high doses which lead to the development of toxicity. After a long period of neglect, there is now significant progress in the development of novel treatment regimens for TB (Podanyn *et al*., 2016).

Difficulties in the Ascertainment of New Drug for TB

The barriers in the ascertainment of novel drugs for TB are various: unavailability of animal models for the testing of new drugs against persistent forms, the consonance of the drug molecule with rifampicin and isoniazid, limited knowledge about the basic biology

of *M. tuberculosis*, sparsity of biomarkers to assess the efficacy of new drugs, a new drug is always tested in combination and not as a single drug and the drug must not inhibit CYP-450 enzyme (Zumla *et al.*, 2012).

Noble Objectives of New Drugs

As described earlier, current drug regime has many defects which have led to the poor patient complaisance. So, our success will rely on the improvement of new anti-TB agents designed to achieve noble objectives like shorten treatment duration, increase adherence by enabling intermittent therapy, introduce agents with novel mechanisms of action to ensure activity against drug-resistant *M. tuberculosis* and Decrease incidence by developing safer, shorter duration treatment regimens (Matteelli*et al.*, 2010).

Table 2. Second line antitubercular drugs and their related resistance mechanisms.

Drug	Mechanism of action	Common mechanism of Resistance	Source
Amikacin/Kanamycin	Protein synthesis inhibition	16S rRNA target site modulation, Increased drug inactivation via over expression of eisaminoglycosideacetyltransferase	Maus *et al.*, 2005
Capreomycin	Protein synthesis inhibition	Cross-resistance with aminoglycosides plus mutation of tlyA which decreases rRNAmethyltransferase activity	Maus *et al.*, 2005
Fluoroquinolones	DNA gyrase and topoisomerase IV inhibitor	Mutations in gyrA and gyrB causing an alteration to DNA Gyrase A/B binding site and increased ABC-type efflux pump expression	Ginsburg *et al.*, 2003 Mitnick *et al.*, 2007
Ethionamide	Mycolic acid biosynthesis inhibition	Mutations in ethA and inhA causing decreased prodrug activation and InhA over expression (cross-resistance with Isoniazid)	Morlock *et al.*, 2003 Johnson *et al.*, 2006

New Drug Molecules Undergoing Clinical Trials

The formidable challenges associated with target-based approaches have made the discovery of high-quality "hit" compounds to feed the front end of the TB drug pipeline critically reliant upon the use of phenotypic screening to identify small molecules that inhibit the growth and/or survival of *Mtb*(Ioerger *et al.*, 2013). The value of this empiric approach is evidenced by the fact that the clinically approved drugs, bedaquiline (Sirturo) and delamanid (Deltyba), and others, such as PA-824 (Pretomanid), PBTZ169, and Q203, which are currently in clinical development, arose through phenotypic screening (Mdluli *et al.*, 2015). Critically, these drugs, drug candidates, and screening hits have also been used to identify a number of new TB drug targets. These include the AtpE subunit of ATP synthase (the target of bedaquiline) (Andries *et al.*, 2005); the decaprenylphosphoryl-β-d-ribose 2-epimerase, DprE1 (the target of PBTZ169) (Makarov *et al.*, 2014); the trehalosemonomycolate transporter, MmpL3 (the target of indolcarboxamides) (Rao *et al.*, 2013); QcrB, a component of the cytochrome bc_1–aa_3 complex (the target of Q203) (Arora *et al.*, 2014); DnaN, the target of griselimycin (Kling *et al.*, 2015)and FadD32, the target of diarylcoumarins (Stanley *et al.*, 2013). By virtue of their novel mechanisms of action, drugs that are active against these targets have the potential to offer new therapeutic options for the treatment of DS- as well as DR-TB. This review surveys new synthetic/natural molecules with antimycobacterial activity such as:

Bedaquiline (TMC 207)

Bedaquiline is a diarylquinoline in nature. They have a long half-life. Currently it is in Phase II but soon to enter Phase III. Their result against susceptible TB patients is great and moreover they does not show any cross resistance to the present drugs in the market. Bedaquiline exhibits a powerful sterilization

effect and they are active against both the fast and slow growing *M. tuberculosis*. Bedaquiline performs their functions by inhibiting bacterial ATP synthase. It is highly powerful as compared to isoniazid and rifampicin. Moreover in the fed conditions, the serum concentration of the drug increases by two fold as compared to fasting condition. Cytochrome P450 3A4 (CYP3A4) enzyme metabolize this drug. Thus the serum concentration of the drug gets reduced up to 50% in presence of rifampicin which is a CYP3A4 inducer (Matteelli *et al.*, 2010).

PA-824, Nitroiimdazoloxazine

PA-824, bicyclic nitroimidazole drug will soon complete Phase II clinical trials. It is effective against both replicating and hypoxic non-replicating Mycobacterium tuberculosis. It's *in vitro* and *in vivo* activity is high and like Bedaquiline they also does not exhibit cross resistance to any other TB drugs available in the market. Nitroimidazole is basically a prodrug which has been found to show good activity against dormant form along with rifampicin, isoniazid and pyrazinamide drugs.

They have a long half-life. Nitroimidazole show their effect by two mechanisms, either by cell wall synthesis inhibition (like isoniazid) or by respiratory poisoning (like cyanide) in the bacterium. However, replacement of isoniazid or pyrazinamide with PA-824 may lead to the relapse of the disease after 6 months (Singh *et al.*, 2008). PA-824 performs its anaerobic activity by releasing Nitric Oxide causing respiratory poisoning. Moreover under hypoxic non-replicating conditions PA-824 effect on the respiratory complex resulting in a rapid drop of intracellular ATP levels (Manjunatha *et al.,* 2009).

Delamanid (OPC-67683)

Delamanid is a dihydro-nitroimidazooxazole derivative. Its phase II study has been successfully completed and has recently entered Phase III clinical study. The drug exhibited excellent *in vitro* activity with no cross resistance with any first line anti-TB drug. The drug also has a long half-life (Matteelli *et al.,* 2010).Delamanid is a pro-drug which gets activated by the enzyme deazaflavin dependent nitroreductase (Rv3547) which is a reactive intermediate metabolite, It performs its action by inhibiting the synthesis of methoxy mycolic acid and ketomycolic acid which are the main components of mycobacterial cell wall. Delamanid should be used as a part of an appropriate combination regimen for pulmonary MDR-TB in adult patients in whom the current approved regimen cannot be used because of resistance or intolerability (Xavier and Lakshman, 2014).

Fluoroquinolones

The fluoroquinolones are also found to have powerful activity against resistant strains. They mainly act by inhibiting DNA gyrase. Currently, two fluoroquinolones, gatifloxacin and moxifloxacin are presently entering Phase III study (Moadebi *et al.*, 2007).

LL-3858

The drug is currently in Phase I. It has a pyrrole alkaloid nucleus. It is also an analogue of Isoniazid. It also exhibited a potential activity against MDR-TB in mice (Moadebi *et al.*, 2007).

Oxazolidinones

Oxazolidinones are synthetic antibacterial compounds which are orally active. Linezolid got approved in 2000 for the treatment of drug resistant gram positive bacteria. Linezolid is a potent Oxazolidinones which can be used for MDR-TB. It performs its action by inhibiting protein synthesis. It mainly interferes with the formation of the initiation complex. It does not show any cross resistance with the first line drugs (Schecter *et al.*, 2010).

The main target of linezolid is bacterial ribosome (23S RNA in 50S ribosomal subunit). It also exhibits a highly potent activity. Sutezolid (PNU-100480) and AZD-5847 are also chemically related to linezolid. In the recent studies sutezolid is found to be more active than linezolid in recent studies. AZD-5847 has also been recognized for its anti-TB properties. The main concern is that it may lead to hematological issues

(Diekema *et al.*, 2001).

Ethylenediamine derivatives

Ethylenediamines derivatives like SQ-109 is a strong antiTB compound whose structure is similar to the drug Ethambutol which is a first line of drug. The mode of action is not yet clearly understood but it has been found to inhibit lipid/cell wall synthesis (Sacksteder *et al.*, 2012).

Rifalazil (KRM-1648)

Rifalazil (KRM-1648) is a rifamycin derivative which is under Phase II clinical trials. Their activity has been found great promising candidate against bacteria in both animal and human models if taken orally (Rothstein *et al.*, 2006).

Caprazamycin-B

Caprazamycin-B is a promising liponucleoside antibiotic which has been developed in Japan. The drug show bactericidal activity specifically against Mycobacterium species including resistant strains. It mostly inhibits the synthesis of mycobacterial cell wall. The therapeutic efficacy was found to be moderate in mice models (Kaysser *et al.*, 2009).

Teixobactin

Teixobactin is a new class powerful antibiotic that kills drug resistant bacteria. It emerges as a promising compound for its activity against drug resistant Gram positive pathogens. Their mechanism of action is unique as it targets the lipid molecules which are required by the bacteria to build their cell walls. It has been reported that teixobactin has such a rapid activity that it does not allow the resistance development in bacteria. Teixobactin has been regarded as the first new antibiotic drug against resistant bacteria for the last 30 years. Its efficacy hasn't been tested in human beings yet but has been found to overcome the development of resistance to the present antibiotic drugs. It strikes multiple targets in the bacteria. Its potent activity has been shown against resistant tuberculosis in rat models. The precursor of peptidoglycan synthesized in the cytoplasm is lipid II and the precursor of wall teichoic acids (WTA) is lipid II. Teixobactin can bind to both of these lipids to form stoichiometric complexes. These complexes serve as an obstruction in the formation of functional cell envelope (Ling *et al.*, 2015).

Thioridazine

Thioridazine is an old drug that has been used for over 35 years to treat psychosis which when used correctly and with care, produces no harm which when used in combination with certain anti-TB drugs, has the potential to cure the TDR TB patient (Amaral, 2013**).** This neuroleptic is thioridazine (TZ) a compound indirectly derived from the first neuroleptic chlorpromazine (CPZ). TZ has been shown to inhibit the *in vitro* replication of all studied strains resistant to INH and Rif (Van-Ingen *et al.*, 2009**)**, to enhance the killing of intracellular MDR TB and XDR TB by non-killing human macrophages, to cure the infected mouse of antibiotic pan-sensitive strains of Mtb and MDR Mtb strains and now, it has been shown to cure XDR TB patients when used in combination with antibiotics to which the strains were initially resistant (Abbate *et al.*, 2012).

Benzothiazinone

Benzothiazinone is an antimicrobial compounds with anti-gyrase activity, benzothiazinone are regarded as a gyrase inhibitor with potent MTB MIC and inhibitory profiles of the gyrase enzyme with a well correlated structural activity relationship and less cytotoxic effect. Moreover, it is believed that this class of compounds has great potential to be developed as an anti-TB drug candidate (Chandran *et al.*, 2015).

Thiazole–Aminopiperidine Hybrid Analogues

Aryl thioamides used to designed a series of ethyl-4-(4-((substituted benzyl) amino) piperidin-1-yl)-2-(phenyl/pyridyl) thiazole-5-carboxylates by molecular hybridization in five different steps. These compounds were evaluated for their *in vitro Mycobacterium smegmatis* (MS) GyrB ATPase assay, *Mycobacterium tuberculosis* (MTB) DNA gyrase super coiling assay, antituberculosis activity and cytotoxicity. Among the twenty four compounds

studied, ethyl-4-(4-((4-fluorobenzyl)amino)piperidin-1-yl)-2-phenylthiazole-5-carboxylate (14) was found to be the promising compound which showed activity against all test with MS GyrB IC_{50} of 24.0 ± 2.1 μM, 79% inhibition of MTB DNA gyrase at 50 μM, MTB MIC of 28.44 μM, and not cytotoxic at 50 μM (Jeankumar *et al.*, 2013).

Lansoprazole

Better antibiotics capable of killing multi-drug-resistant *Mycobacterium tuberculosis* are urgently needed. Despite extensive drug discovery efforts, only a few promising candidates are on the horizon and alternative screening protocols are required. Here, by testing a panel of FDA-approved drugs in a host cell-based assay, we show that the blockbuster drug lansoprazole (Prevacid), a gastric proton-pump inhibitor, has intracellular activity against *M. tuberculosis*. *Ex vivo* pharmacokinetics and target identification studies reveal that lansoprazole kills *M. tuberculosis* by targeting its cytochrome bc_1 complex through intracellular sulfoxide reduction to lansoprazole sulfide. This novel class of cytochrome bc_1 inhibitors is highly active against drug- resistant clinical isolates and spares the human H^+K^+-ATPase thus providing excellent opportunities for targeting the major pathogen *M. tuberculosis*. Lansoprazole belongs to a class of drugs known as "proton-pump inhibitors" that keep the stomach from pumping too much acid, thus preventing heartburn and ulcers. "Proton-pump inhibitors are both safe and widely sold around the world," says Stewart Cole. "Being highly active against drug-resistant

strains of *M. tuberculosis*, this novel class of drugs provides us with an excellent opportunity to treat tuberculosis (Rybniker *et al.*, 2015).

VCC234718

A new molecule, VCC234718, has been found with promising antitubercular activity and its toxicity in mammalian cell is limited. By studying the chemical biology, enzymology, and structural biology, it was found that the target of VCC234718 in *Mtb* is the inosine-5′-monophosphate dehydrogenase (IMPDH), GuaB2, an enzyme that catalyzes the NAD^+-dependent conversion of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) in the de novo purine biosynthesis pathway. Moreover it has been found that GuaB2 depletion is bactericidal in *Mtb* in vitro, in macrophages, and in mouse lung. All these evidences shows that GuaB2 is a new TB drug target (Singh *et al.*, 2017).

Mycobacterium indicuspranii *(MIP or Mw)*

Worldwide tuberculosis is among major health problem. In order to control tuberculosis, Bacillus Calmette–Guerin (BCG) has been used as a vaccine, but its effective value has been continuously variable among different populations of the world. From the past three decades, the potential of protective efficacy of *Mycobacterium indicuspranii* (MIP or Mw) against mycobacterial infections (leprosy and tuberculosis) has been studied by several workers. The MIP has been used as an immune-prophylactic tool and also as an accessory to chemotherapy. The present day study, reports the beneficial effect of prior immunization with MIP. It not only results in the improvement of histopathological condition but also reduces the bacterial burden, as an augmenter to the effects of chemotherapy in experimental tuberculosis (Rawat *et al.*, 2016).

HVJ-liposome/ or HVJ-envelope/HSP65 DNA+ IL-12 DNA

Masaji OKADA (Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center) have developed a novel tuberculosis (TB) vaccine (HVJ-liposome/ or HVJ-envelope/HSP65 DNA+ IL-12 DNA). The vaccine provided remarkable protective efficacy in mouse compared to BCG vaccine, and improved the histopathological tuberculosis lesions. This vaccine also exerted therapeutic effect in vivo against XDR-TB as well as drug-sensitive TB in mice. Furthermore, by using the cynomolgus monkey (similar to human tuberculosis), this novel vaccine provided higher protective efficacy (mortality) than BCG mortality. Furthermore, the combination of HSP65+IL-12/HVJ and BCG by the

priming-booster method showed a synergistic effect in the TB-infected cynomolgus monkey (100% survival). These data indicate that novel DNA vaccine might be useful against TB for human clinical trials (Okada and Kobayashi, 2007).

Pyridomycin

Scientists report that a soil bacterium secretes a natural product called pyridomycin. This new drug shows promising result for the treatment of tuberculosis. Pyridomycin is a natural antibiotic produced by the bacterium *Dactylosporangiumfulvum*. Many drug-resistant types of tuberculosis bacterium that no longer respond to the front-line drug isoniazid are the target of this drug (Ruben *et al*., 2012).

Sansanmycin

Sansanmycin, a nucleosidyl-peptide antibiotic, was isolated from an unidentified *Streptomyces* sp SS. The structure of Sansanmycin was elucidated by analyses of its alkaline hydrolysate and spectroscopic analyses. Sansanmycin exhibits antibacterial activity against *Mycobacterium tuberculosis and Pseudomonas aeruginosa* with MIC values of 10 and 12.5 mg/ml, respectively (Anh *et al*., 2017).

Artemisinin

Artemisinin is the ancient way to prevent the ability of Mycobacterium Tuberculosis to become dormant. The dormancy of mycobacterium is beneficial in way as the antibiotics are ineffective in this state. In order to thrive in the body Mycobacterium needs Oxygen. Our immune system deprives this bacterium from oxygen so that its infectious ability can be checked. Artemisinin attacks a molecule called heme in Mycobacterium that acts as an oxygen regulator. Disrupting the heme and by essentially turning it off by Artemisinin the mycobacterium wouldn't be able to detect how much oxygen it is getting for infection. When the Mtb is deprived of oxygen, it goes into a dormant state, which protects it from the stress of low-oxygen environments (Zheng *et al*., 2016).

Innovative drug delivery vehicles for Anti-TB drugs

Different approaches of drug delivery also regulate the efficacy of a treatment. As a result of this, the use of nanotechnology-based therapy has been researched over the past few years for substitute the administration of antibiotics in the free form with an access using drugs that are encapsulated with nanoparticle (Griffiths *et al*., 2010). The formulations such as liposomes, solid lipid nanoparticles, nanoemulsions, polymeric micelles are gaining utmost importance due to their potential advantages to target the drug to specific site (Paranjpe *et al*., 2014).

Nanotechnology and Anti-TB Drugs

Currently, nanotechnology based pulmonary drug delivery systems are gaining utter importance due to their numerous advantages like large surface area for absorption, high permeability and good blood supply. Recent researches have been shown that alveolar epithelium serve as a prominent site for the absorption of various therapeutics, as a result of this development, the technologies for drug targeting to alveoli are achieving a great attention (Pastoriza *et al*., 2014).

Nanoparticles

Basic Characteristics and Advantages

Nanoparticles used as drug delivery vehicles are defined as submicron (<1μm) colloidal particles. This definition includes nanospheres in which the drug is adsorbed, dissolved, or dispersed throughout the matrix and nanocapsules in which the drug is confined to an aqueous or oily core surrounded by a shell-like wall. Alternatively, the drug can be covalently attached to the surface or into the matrix (Kreuter, 2004). Nanoparticles are made from biocompatible and biodegradable materials such as polymers, either natural (e.g., gelatin, albumin) or synthetic (e.g., polylactides, polyalkylcyanoacrylates), or solid lipids. In the body, the drug loaded in nanoparticles is usually released from the matrix by diffusion, swelling, erosion, or degradation (Muller *et al*., 1995).The important technological advantages of nanoparticles as drug delivery vehicles include high constancy/long shelf life, high carrier capacity (i.e.,

many drug molecules can be incorporated in the particle matrix), Increased bioavailability (slow, sustained, and controlled drug release), feasibility of incorporation of both hydrophilic and hydrophobic substances, viability of variable routes of administration, including oral administration and inhalation and Minimal side effects compared to conventional drugs and improved compliance.

Liposome-Based Drug Delivery Systems

Liposomes are pint-size closed vesicles consisting of phospholipid bilayer enfolding an aqueous section. Their ability to encapsulate both hydrophilic and hydrophobic drugs made them a promising drug delivery model for bioactive compounds. In order to access better chemotherapeutic efficacy in animal models like mice, liposomes have been evaluated for the constant delivery of anti-TB drugs (Khuller *et al.*, 2004; Moretton *et al.*, 2010). Some drugs like doxorubicin for breast cancer and amphotericin B for fungal infection have been approved for human use (Davis *et al.*, 2008). As liposomes are susceptible to intestinal lipases, they must be administered by either respiratory means or intravenous route. Nonspecific uptake by mononuclear phagocyte system (MPS) of liver and spleen can be reduced by the inclusion of PEG in the liposomal formulations (Deol and Khuller, 1997). When INH and rifampin encapsulated in the lung specific stealth liposome were used against Mtb infection, it was revealed that liposome encapsulated drugs at and below therapeutic concentration was more effective than free drugs against TB (Deol *et al.*, 1997).

Niosomes-Based Drug Delivery Systems

Like liposome, niosomes colloidal particles are thermodynamically stable formed by self-assembly of nonionic surfactants and hydrating mixture of cholesterol in aqueous medium resulting in multilamellar systems, unilamellar systems, and polyhedral structures (Uchegbu and Vyas, 1998).

A niosomal drug delivery system of antitubercular agents such as isoniazid is formulated that has exceptional potential for development into a low dose performed with effective treatment for tuberculosis (Karki *et al.*, 2008). Niosomes of rifampicin and gatifloxacin are being prepared by lipid hydration technique. The bactericidal activities of the niosomal formulation have also been reported by the BACTEC radiometric technique using the resistant strain (RF 8554) and sensitive strain (H37Rv) of *Mycobacterium tuberculosis* which showed inhibition and reduced growth index. This means that rifampicin and gatifloxacinniosomes provided extensive release of drugs, which was optimum to provide a decreased dose, fewer days of treatment, and more patient compliance (Rani *et al.*, 2010). Niosomal encapsulation of pyrazinamide has also been done in order to achieve sufficient macrophage targeting and to overcome drug resistance (El-Ridy *et al.*, 2011). Thomas and Bagyalakshmi concluded that all three polymers such as Brij-35, Tween 80, and Span-80 used for the successful formulation of pyrazinamide niosomes helped avoid hepatotoxicity by keeping the cholesterol content constant. It was seen that Span-80 formulation had the highest percentage release when compared to other formulations (Thomas and Bagyalakshmi, 2013).

Microemulsion-Based Drug Delivery Systems

Danielsson and Lindman have correctly defined microemulsion as a system of water, oil and an amphiphile (surfactant and co-surfactant) which is a single optically isotropic and thermodynamically stable liquid solution (Danielsson and Lindman, 1981). Because of their thermodynamic stability, high diffusion and absorption rates, ease of preparation, and high solubility, in recent years, microemulsions have gained a lot of attention for the development of new drug delivery vehicles (Sarciaux *et al.*, 1995).

Tween-based microemulsion systems can also be for potential application as a drug carrier for the anti-TB drug rifampicin. They formulated microemulsion composed of oleic acid + phosphate buffer (PB) + Tween 80 + ethanol and examined its potential as a delivery system for an antitubercular drug. They studied numerous structural features with various physiochemical methods such as electron microscopy,

NMR, optical microscopy, and dissolution and release kinetics and concluded that microemulsions containing Tween 80 were successful, since they encapsulated the anti-TB drugs (RIF, INH, and PZA) in different combinations by means of conductivity and viscosity, with no precipitation or phase separation (Mehta *et al.*, 2007). Ahmed *et al.* developed nanoemulsions of rifampicin which indicated a great potential of intravenous delivery of this antitubercular drug (Ahmed *et al.*, 2008). In another study, Kumar performed the inclusion of INH in O/W microemulsion or W/O microemulsion comprising TX100: AcOH (1:1), followed by cetyltrimethylammonium dichromate (CTADC), chloroform, and water; this microemulsion system presents the opportunity of sustained release, increasing drug solubility and bioavailability (Kumar, 2011).

Conclusion

Despite the challenges outlined we firmly believe the field of tuberculosis drug discovery is much better placed to achieve significant therapeutic advances in the coming decade. It can be done by better understanding of Mtb life cycle, the pharmacological requirements for successful Mtb drugs, and the new antitubercular chemical matter derived in recent years. The importance of recent improvements in animal infection models that better mimic the pathology of the human tuberculosis lung, coupled with new imaging technologies, provide us with much better predictive preclinical models to produce drug combinations efficacious against hard to treat slow growing sub-populations, should not be understated.

Finally, we believe there is much strength in the expertise, experience and diversity of investigators currently working in this area, which needs to be nurtured to continue the important fight against MDR-TB. Although identifying novel anti-TB agents remains a priority, the development of the nanoparticle-based delivery systems for currently used agents may represent a cost-effective and promising alternative. It can be expected that future research will concentrate on the development of the vectorized delivery systems combining advantages of the colloidal carriers, such as large payloads of a drug, with active targeting to the infection sites. Moreover, development of innovative formulation technologies suggests that nanoparticles can be incorporated into various solid dosage forms (microparticles, granules, or tablets), which can release the nanoparticles at the site of action, preserving their original properties. These approaches would further improve efficacy and practicability of the nanoparticle-based formulations.

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