Present Status of Nanoparticle Research for Treatment of Tuberculosis

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ABSTRACT - Nanotechnology has offered enormous improvement in field of therapeutics by means of designing of drug delivery systems and opened the possibility of controlling infections at the molecular level. Nanocarriers can cross biological barriers and are able to target cellular reservoirs of Mycobacterium tuberculosis (M. tuberculosis). Nanoparticle-based systems have significant potential for treatment and prevention of tuberculosis (TB). A variety of nanocarriers have been widely evaluated as potential drug delivery systems for various administration routes. Targeting the drugs to certain physiological sites such as the lymph nodes has emerged as a promising strategy in treating TB with improved drug bioavailability and reduction of the dosing frequency. Nanotechnology based rational targeting may improve therapeutic success by limiting adverse drug effects and requiring less frequent administration regimes, ultimately resulting in more patients compliance and thus attain higher adherence levels. The development of nanoparticle based aerosol vaccine is undergoing which could serve as new platform for immunization. Present article compiles the general physiological aspects of the infection along with new research uptades on nanocarriers used in prevention of tuberculosis.

INTRODUCTION

In year 1993, World Health Organization (WHO) declared tuberculosis as global emergency. Tuberculosis (TB) is a highly contagious persistent infection caused by Mycobacterium tuberculosis and Mycobacterium bovis and has highest mortality rate than any other infectious disease. Worldwide One-third of the population is infected and of these, 8 to 10 million develop active disease and 2 million die each year and serve as world’s second most common cause of death after HIV/AIDS. Presence of immunosuppressive condition like diabetes, alcoholism, malnutrition, chronic lung disease & HIV/AIDS may increase the chances of tuberculosis infection [1, 2]. The disease presents mainly in the lungs but can also develop as extrapulmonary tuberculosis in the central nervous or circulatory systems or elsewhere in the body. Untreated active tuberculosis has a mortality rate of approximately 50% [3-5].

Tubercle bacilli are slender, acid-fast, non-motile gram-positive bacilli. TB bacteria remain viable in the air for a long time, eventually they get inhaled by lungs and engulfed by alveolar macrophages (white blood cells) where they start to replicate within 2 to 3 weeks [6]. In 95% of cases, the macrophages throughout the body are able to carry the bacteria without any apparent disease sign. However, if the bacteria are not completely destroyed, they remain dormant for several days and may reactivate years later.

Treatment of active TB is complex and is becoming more and more complex with the emergence of multidrug-resistant tuberculosis (MDR) and HIV infection. Available TB treatment involves daily administration of four oral antibiotics for a period of six months or more [7]. Due to the high percentage of side effects (like ototoxicity and nephrotoxicity) and the extended duration of treatment results in low patient adherence [8]. History of tubercular chemotherapy started with discovery of streptomycin in 1944 [9]. Furthermore, only a small fraction of the anti-tubercular drugs reach an alveoli which is the desired site of drug action. The development of a tubercular vaccine has different array of challenges. The bacteria are carried to lymph nodes where they are surrounded by lymphocytes in a granuloma that can persist for years, and in coming years turn in active and infectious disease state.

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A successful tuberculosis vaccine must be able to induce a variety of T cell responses, including Th-1 immune response [10, 11]. The currently used Bacillus Calmette-Guerin (BCG) vaccine offers extremely limited protection. However, several other Mycobacterium tuberculosis antigens have been identified for potential use as vaccine antigens, including the three protein Antigen 85 complex (Ag85A, Ag85B and Ag85C), the surface exposed lipoproteins PstS (PstS-1, PstS-2 and PstS-3) and the early secretory antigenic target protein ESAT-6 [12-14]. Cationic liposomes entrapped antigenic tuberculosis ESAT-6 protein and complexed with TLR agonists were evaluated for use as a prophylactic vaccine system by Zaks research group [15]. They found to have considerable protective immunity after an aerosol challenge with virulent Mycobacterium tuberculosis [16]. Nanoscale aerosol vaccines for tuberculosis have also been developed which can perfuse throughout the respiratory pathway and also increase the amounts of drug reaching to the target alveoli. Garcia Contreas et al. have recently synthesized a particle system with both micrometer and nanometer dimensions for aerosolized delivery of the attenuated tuberculosis vaccine, BCG [17]. These dried “nanomicroparticle” vaccines were synthesized with two axes with nanoscale dimensions and a third axis with a microscale dimension. Aerosol delivery of BCG encapsulated nanomicroparticles in guinea pigs enhanced their resistance to tuberculosis infection, and generated better immune protection than a standard parenteral BCG formulation. Alternatively, the nanoparticulate delivery of aerosolized IFN-γ via the pulmonary route has been shown to be a safe and efficient new adjunct treatment for tuberculosis [18, 19] when used in combination with antibiotics. The particle size-dependent cell trafficking can greatly influence particulate strategies for vaccination for mucosal or parenteral delivery [20].

Since last two decades, pharmaceutical literature discusses various aspects of drug targeting and problem associated with it. Different nanoparticulate strategies are developed to specifically deliver chemotherapeutic compounds to target disease sites. They increase drug’s therapeutic index by strictly localizing its pharmacological activity to the site or organ of action. The use of nanoparticulate drug delivery
system can improve tolerability of toxic chemotherapeutics and eventually bioavailability. The particle size between 50 to 200 nm is desired for maximized drug localization upon administration by inhalation. Lung has a larger surface of absorption and a thin alveolar epithelium [21]. In lungs, particle deposition takes place by inertial impaction, sedimentation and diffusion [22, 23]. Large particles (>5 μm) tend to deposit by impaction in the extra thoracic cavities, particles (1-5 μm) deposit deeper in the lungs by inertial impaction and sedimentation while very small particles (<1 μm) are driven by diffusion and mostly remain suspended are exhaled [24]. Nanosized particles are promising candidate as they are slowly cleared from lungs and can escape both phagocytic and mucociliary clearance. The high lung bioavailability and rapid drug absorption through the pulmonary epithelia enable the lowering of drug doses in parallel maintaining therapeutic concentration. A smaller dose combined with the absence of first pass metabolism and avoidance of gastrointestinal track is expected to lead reduced systemic side effects and enhanced tolerability. Various Anti-TB drugs have been formulated in dry microparticles for pulmonary delivery, including capreomycin [25, 26] and para-aminosalicylic acid [27]. They provide promise in targeting TB sites, that can be directly administered to the lungs with reduced systemic side effects [28].

Various nanoparticulate systems includes, polymeric nanoparticles, lipid nanoparticles, nanosuspensions, nanoemulsions etc. and can be used to treat several types of parasitic infections [29-33]. Obviously it is very important to understand nature of biological and cellular membranes at target site, distribution and presence of drug receptors and enzymes responsible for drug metabolism. Versatility of nanoparticulate carriers also enables tailoring of drug delivery systems according to the target nature, desired pharmacokinetic profile and route of administration with reduced side effect. In present review uses of such carrier systems and their potential advantages is highlighted in tubercular chemotherapy [7, 34].

Background

Nanoparticles are defined as submicron (<1μm) colloidal particles. Monolithic nanoparticles (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 [35]. Alternatively, the drug can also be covalently attached to the surface or dispersed into the matrix. Nanoparticles are made from biocompatible and biodegradable materials such as polymers, either natural (e.g., gelatin, albumin) or synthetic (e.g., polylactides, polyalkylycnoacrylates), or solid lipids (SLN®, NLC®) [36] in the body, the drug loaded in nanoparticles is usually released from the matrix by diffusion, swelling, erosion, or degradation.

The various advantages of nanoparticles as drug carriers include high stability (i.e., long shelf life); high carrier capacity (i.e., many drug molecules can be incorporated in the particle matrix); feasibility of incorporation of both hydrophilic and hydrophobic substances; and feasibility of variable routes of administration, including oral administration and inhalation. These carriers can also be tailor made to enable controlled (sustained) drug release from the matrix [37]. In general, the uptake of nanoparticles occurs by three mechanisms (1) transcytosis (2) intracellular uptake and transport via the epithelial cells lining the intestinal mucosa, (3) uptake via peyer's patches [38, 39].

Drug delivery explored in TB chemotherapy

Liposomes

Liposomes are small spherical vesicles formed of amphipilic lipids enclosing an aqueous core. They are widely studied as carrier systems for hydrophilic drugs [40, 41]. Gentamicin incorporated liposomes were evaluated for antibacterial activity in M. avium infected mouse model. The drug encapsulated liposomes significantly reduced the bacterial count in spleen and liver as compared to free drug. A dose-related reduction in bacterial load was observed, but no sterilization was found [42]. Similar results were reported in literature for second-line antibiotics liposomes [43-45]. Deol and Khuller developed Stealth® liposomes for the targeted delivery of anti-TB drugs to the lung. Liposomes were composed of mixture of phosphatidylcholine, cholesterol, dicetylphosphate, O-steroyl amylopectin and monosialogangliosides / distearyolphosphatidylethanolaminepoly (ethylene glycol) 2000. In vivo biodistribution after intravenous administration in healthy and tuberculosis infected mice showed pronounced increase in accumulation from 5.1% for conventional liposomes to 31% for PEGylated liposomal systems after 30 min. The accumulation extent was mainly associated to the composition of the liposomal vesicles. Drug
uptake levels in the lungs increased to approximately 40% for the PEGylated nanocarriers when administered to pretreated infected animals only after 30 min. whereas, 30–50% reduction in uptake was observed for modified liposomes for accumulation in the liver and spleen [46]. Drug-loaded nanocarriers showed a significant decrease in the toxic effects when evaluated for cytotoxicity in peritoneal macrophages compared to free drugs.

A 12 mg/kg dose of liposomal drug and free (isoniazid) INH reduced the number of CFU (colony formation units) in the lungs to about 3.9 and 4.5 log units, respectively whereas at 10 mg/kg, 3.8 and 4.3 log units, respectively. Decrease in CFU was observed in similar pattern for liver and spleen. In addition, administration of sub-therapeutic doses also (4 and 3 mg/kg for INH and RIF (Rifampicin) respectively) led to a sharp decrease in CFU compared to the free drugs. The therapeutic activity of free and encapsulated INH and RIF was evaluated at both therapeutic and sub-therapeutic dose [47]. Parenteral clofazimine liposomes were [48] evaluated in acute and chronic murine infections [49]. Liposomes significantly reduced the toxicity of the drug both in vitro and in vivo and showed enhanced anti-TB activity in acute and chronic models. Administration of encapsulated drug to chronically infected mice showed total clearance of bacilli from the liver and spleen. Recent investigations reports, PYZ [50] and rifabutin containing liposomes [51].

**Nanoemulsions**

Nanoemulsion, many times referred as mini-emulsions or sub-micronemulsions by dispersing mainly oil in water. Thermodynamically stable nanoemulsion (mean particle size of 80.9 nm and polydispersity index of 0.271) of ramipril, were developed for oral administration. In vitro drug release showed that drug release till 24 h from nanoemulsion and was highly significant (p<0.01) as compared to marketed capsule formulation and drug suspension. The relative bioavailability of ramipril nanoemulsion to that of conventional capsule was 229.62% and to drug suspension was 539.49 suggesting the use of developed ramipril nanoemulsion for pediatric and geriatric patients [52]. In another study, they investigated effect of Labrasol on self-nanoemulsification efficiency of ramipril nanoemulsion [53]. Ahmed et al., developed various parenteral o/w nanoemulsions of RIF (47 and 115 nm) using GRAS listed excipients (US-FDA). The entrapment efficiency was found to be 99% with excellent stability over 3 months with slight increase in particle size and showed initial burst drug release of 40 to 70% after 2 h [54]. Mehta et al. performed physico-chemical analysis of INH microemulsions and confirmed the release of drug from microemulsion was non-Fickian [55]. In another study, the same group studied the changes in the microstructure of Tween 80-based microemulsion in the presence of anti-TB drugs viz. INH, PYZ (pyrazinamide), and RIF [56].

**Polymeric nanoparticles**

In Polymeric nanoparticles, the drug is attached, entrapped or encapsulated in polymeric core and depending upon the method of preparation, they are called as nanoparticles, nanospheres or nanocapsules [57]. Polymeric nanoparticles represent an attractive alternative to liposomes [58]. Pandey et al., [59] developed sustained release RIF, INH and PYZ loaded poly(lactide-co-glycolide) (PLG) nanoparticles for oral delivery. In mice, the drugs could be detected in the plasma up to 4 days for RIF and 9 days for INH and PYZ; therapeutic concentrations in the tissues were detected till 9 to 11 days after single oral dose administration of nanoparticles whereas free drugs after administration were cleared within 12 to 24 h from the plasma.

Five oral doses every 10 day of nanoparticles were sufficient to achieve complete bacterial clearance from the organs of TB bacillus infected mice, whereas free drugs took 46 doses to get the same results. Nanoparticles showed similar results when tested in guinea pigs [60]. In parallel, microparticles were tested and found to be less effective as compared to nanoparticles [61, 62]. In order to enhance efficiency of these nanoparticles, the surface was modified using wheat germ agglutinin [63]. Presence of lectins on surface showed improved mucoadhesion thereby faster biorecognition of carriers by glycosylated structures in the intestine and prolonged serum half life [64]. Upon oral administration in mice, these nanoparticles showed prolonged serum levels of 6-7 days for RIF, 13-14 days for INH and PYZ as compared to uncoated nanoparticles which showed 4-6 days for RIF and 8-9 days for unmodified nanoparticles. Surface grafted nanoparticles showed detectable drug levels in lungs, liver, and spleen up to 15 days. Three oral doses of coated nanoparticles administered every 14 days (Vs. 45 daily doses of free drugs) were sufficient to give complete bacterial clearance.
Anti-TB drugs encapsulated in nanoparticles were studied by administration via pulmonary route [65]. In guinea pigs, a single nebulization of RIF, INH, and PYZ encapsulated nanoparticles generated same effect as that of oral administration, i.e. sustained therapeutic drug levels in the plasma and in lungs was up to 6 to 8 days and 11 days, respectively. After pulmonary administration, complete sterilization in lung was observed only after administration of five doses at every 10th day whereas to generate same effect orally 46 daily doses were required agglutinin surface modified nanoparticles required only three doses administered each after 14 days for 45 days. Passive targeting of chemotherapeutic compounds to alveolar macrophages has also been explored using pulmonary administration of nanoparticles for the treatment of tuberculosis infections [66-68]. Flexibility of the nanoparticle-based formulations was further demonstrated by effective subcutaneous treatment of mice infected with M. tuberculosis [69]. A single subcutaneous dose of RIF, INH, and PYZ loaded poly-(dl-lactide-co-glycolide) PLG nanoparticles maintained sustained therapeutic drug levels in plasma for 32 and 36 days in lungs/spleen and resulted in complete clearance of bacteria as compared with pure drug which required daily treatment (35 oral doses).

Econazole and moxifloxacin encapsulated nanoparticle were prepared by the multiple emulsion and solvent evaporation technique. Potential of developed nanoparticles was evaluated individually and in combination in murine mice model in order to develop a more potent orally administered regimen for TB. Econazole and moxifloxacin loaded PLG nanoparticles showed prolonged plasma levels up to 5 and 4 days, respectively. The drugs levels in lungs, liver and spleen were lasted till 6 days as compared to pure drugs which cleared within 12-24 h. In M. tuberculosis infected mice, only 8 doses of polymeric nanoparticles individually were sufficient to suppressed bacterial clearance where as pure drug required 56 doses daily of moxifloxacin and 112 doses twice a day of econazole. Whereas combination of two drugs proved to be significantly efficacious compared with individual drugs. Furthermore, addition of third drug RIF to this combination showed complete bacterial clearance within 8 weeks. Thus, suggesting use of developed carriers for intermittent tubercular chemotherapy [54].

Anisimova et al. prepared RIF, INH and streptomycin loaded PBCA (poly(n-butylcyanoacrylate)) and PIBCA (poly(isobutylcyanoacrylate)) nanoparticles and studied in-vitro cellular uptake in human blood monocytes in order to develop drug depot system. Promising enhancement in intracellular concentration to the extracellular concentration for encapsulated INH (4-8 fold), streptomycin (7 fold) and RIF (22-25 fold) was observed as compared to free drug INH (similar), streptomycin (undetectable) and RIF (5 times) [70].

In another work, moxifloxacin-loaded PBCA nanoparticles (418 nm) were prepared by anionic polymerization of poly(butyl-2-cyanoacrylate). In vitro drug release for formulation containing encapsulated moxifloxacin showed initial burst release followed by sustained drug release of 65% at end of 48 h.

Moxifloxacin loaded NPs were more toxic than free drug when subjected to cytotoxicity assay. Cellular uptake to infected cells showed pronounced increase (2-3 fold) in the intracellular drug concentration. After IV administration, anti-TB activity in mice infected with M. tuberculosis showed a significant decrease in the total mycobacterial count in the lungs suggesting potential of encapsulated moxifloxacin to kill intracellular bacilli compared to free drug [71].

Injectable PLGA (poly(lactic-co-glycolic acid)) nanoparticle-based implants were also administered subcutaneously in a murine model. A single subcutaneous dose could able to maintain drug levels in plasma, lungs and spleen for < 1 month and bacterial counts remain almost undetectable in these organs [69]. Zahroor et al. used ionotropic gelation method to prepare alginate nanoparticles (235 nm diameter) of anti-TB drug. Followed by oral administration to mice, Free drugs were cleared within 12 to 24 h from blood but were detectable in tissues (e.g. spleen, liver and lung) till next day. Whereas polymeric nanoparticles were detected in plasma up to 7 for ETB (ethambutol), 9 for RIF, 11 for INH and PYZ. The drug levels in organs were detectable in tissues till 15th day [72].

Plasma life of lectins grafted PLGA nanoparticles were substantially extended to 6–14 days in mice as compared to uncoated (4–9 days) after oral and inhalation administration. Moreover, complete bacterial clearance was achieved in various organs (lungs, liver and spleen) only after 3 doses with frequency of 1 dose after each 14 days [60]. du Toit et al. developed emulsion-based polymeric nano-formulations (77–414 nm) of INH-loaded using
nanoprecipitation technique. Two kinds of precursors were used: water- or emulsion-based systems. The size of the nanoparticles can be adjusted by changing the polymer concentration; lower polymer concentration resulted in smaller particles. *In vitro* release studies indicated initial burst release till 2 h depending upon the technique used for preparation of nanoparticles [73].

**INH implants** after one time implantation were studied in mice by Gangadharam et al. [74]. Similarly, PYZ three times the daily dose of single PLGA polymer implant showed sustained levels up to 54 days. When investigated for chemotherapeutic efficacy showed single implant was similar to standard oral treatment with drugs (INH & PYZ) given daily for 8 days. Kailasam and coworkers also studied implantable PLGA rod in rabbit, prepared implants could able to maintain drug levels up to 63 days [75]. Urine samples collected after 6 weeks inhibited the growth of M. tuberculosis *in vitro* when measured using radiometric assay. But these type of delivery systems generally require surgical operation, and has disadvantage of immobilization. Reddy et al. showed that intradermally administered, ultra-small (25 nm diameter) nanoparticles were more efficient at interstitial lymphatic trafficking to lymph node resident dendritic cells than larger (100 nm diameter) nanoparticles [76]. Song et al., fabricated silver nanoparticles (< 10 nm) and studied their antimicrobial mechanism. Prepared nanoparticle showed excellent antimicrobial property for nanosilver particles [77]. Pandey et al. studied the pharmacokinetics and antibacterial effect of drug loaded nanoparticles after pulmonary administration in guinea pigs using compressor nebulizer system [59].

**Solid lipid nanoparticles (SLN)**

In SLN, the drug is mainly entrapped in solid lipid matrix to produce lipid nanoparticles of size range 50-1000 nm and they produced using hot or cold high pressure homogenization technique. It is noteworthy that the solid lipid nanoparticles display important advantages, such as the composition (physiologic compounds) and the possibility of large-scale production favored by the feasibility to avoid organic solvents in the manufacturing process [78]. A sterilizing effect was achieved after administration of solid lipid nanoparticles [79]. No tubercle bacilli could be detected in the lungs/spleen after seven doses of treatment of infected guinea pigs with drug-loaded solid lipid nanoparticles. Pandey R., developed RIF, INH and PYZ loaded SLN by using emulsion solvent diffusion technique and tested against experimental tuberculosis. SLN formulations following a single oral administration to mice maintained therapeutic drug concentrations in plasma till 8 days and in the organs rich in MPS (lungs, liver and spleen) for 10 days as compared to free drugs which were cleared within 1–2 days. In M. tuberculosis H37Rv infected mice, 5 oral doses at every 10th day of drug loaded SLNs were sufficient to completely suppress bacterial load in the lungs/spleen whereas free drug required administration of 46 daily oral doses to get same effect. SLN incorporated antitubercular drug significantly reduced the dosing frequency and improved bioavailability [79].

**Nanosuspensions**

Nanosuspensions, poor water soluble drugs are dispersed in aqueous phase containing stabilizing agent. Presently more than 8 candidates are in clinical trials [80]. Another drawback to the low aqueous solubility is the inability to conduct preliminary biological and clinical evaluations of new drug candidates [81]. Clofazimine, a riminophenazine compound, is an agent considered for treating patients with M. avium infection. However, use of this drug was restricted because of its poor solubility. Clofazimine was formulated as a nanosuspension (385 nm) and administered to mice intravenously. It resulted in a considerable reduction of bacterial loads in the liver (72.5 mg/kg tissue), spleen (81.4 mg/kg tissue), and lungs (35.0 mg/kg tissue) of mice infected with M. avium [82], when compared with pharmacokinetic data, drug concentrations in these organs reached high concentrations, well in excess of the minimal inhibitory concentration for most M. avium strains. The effects of clofazimine nanocrystals were comparable to those of the liposomal formulation used as a control in this study. This study was specially planned to overcome the poor solubility and toxicity. Reverchon et al. developed RIF sub micronic particles (400 nm to 3 μm) using supercritical carbon dioxide-assisted atomization suitable for parenteral and aerosolizable drug delivery systems. They studied effects of various solvents on resultant particle size and drug degradation suggesting use of nanoparticle production approach for more convenient TB pharmacotherapy administering drug locally to the lungs [83, 84].
Hulda Swai’s (CSIR’s Centre for Polymer Technology, South Africa) team have developed nanoparticles (200 nm) of four frontline anti-TB drugs aiming to shorter treatment regimen with single dose drug application that will last for several days or weeks. They showed that the nanoparticles release the drugs into the bloodstream at a slower rate and for a more prolonged period (up to ten days) as compared to conventional therapy [85].

**Micelles**

Micelles are submicroscopic aggregates (20-80 nm) of surfactant molecules resulting in liquid colloid. Jiang et al., synthesized thermo-responsive poly(ε-caprolactone-coglycolide)–poly(ethylene glycol)–poly(ε-caprolactone-coglycolide) (P(CL-GA)–PEG-P(CL-GA)) block copolymers having micelle-forming and gelation properties. They can be used for development of drug depot system. The sol–gel transition temperature was optimized by selecting optimized GA/CL ratio and length of the hydrophobic segments. RIF sustained release was obtained over 32 days from 25% gel matrix [30].

INH-poly(ethyleneglycol)–poly(aspartic acid) conjugates were studied for sustain release of the drug. A 5.6-fold increase in anti-tuberculosis activity against M. tuberculosis was found for micelle-forming prodrug as compared to the free drug [86]. Similar attempts were made to incorporate PYZ and RIF in micelles ( <100 nm) aiming to minimize renal filtration and prolonging mean residence times in the blood stream with improved antimicrobial activity [87, 88]. INH lipid derivatives was designed by Jin et al. to reduce the resistance. The new amphiphilic molecules resulted in formation of monolayers at the air/water interface. Flexible medium-long tails formed self assembling nano-sized vesicles whereas short lipid tail-derivatives resulted in weak hydrophobic interactions. Lipid vesicles showed increased penetration of the drug into the pathogen leading to promising antibacterial activity against Mycobacterium [89].

Solubilization of RIF within polymeric micelles of various linear and branched PEO–PPO was studied and found to increased about 2 fold depending upon RIF entrapment. Other studied amphiphilic block copolymers of different
molecular weight further improved the solubilization of RIF by 5 to 7 fold [90]. Furthermore, drug-loaded stereocomplex micelles were developed by Chen et al. using the specific assembly of enantiomeric poly (ethylene glycol)– poly(L-lactide) (MEPG-PLLA) and poly(ethylene glycol)-poly(D-lactide) (MEPG-PDLA) block copolymers (1:1 ratio of L-PLA- and D-PLA-containing block copolymers). An increase in the length of the PLA segment resulted in lower CMC values and larger nano-aggregates. Developed stereocomplexes showed improved RIF loading and encapsulation efficiency than enantiomerically pure micelles. Stereocomplex released $t_{50\%}$ between 4-8 hours and $t_{100\%}$ after 48 h, which can be further manipulated according to polymer molecular weight [91]. PLA-modified chitosan oligomers spherical micelles (154 to 181 nm) were prepared by Wu et al. Developed RIF chitosan oligomer micelles showed initial burst drug release of 35% within 10 h followed by more sustained drug release till 5th day suggesting suitability of carrier for prolonged anti-TB activity with reduced toxic effects [92].

Pulmonary tuberculosis causes lung fibrosis and alveolar collapse due to a dysfunction of pulmonary surfactant. Nanoparticles of pulmonary surfactant (surface tension of approx. 25-30 mN/m) can be employed as delivery agents for the anti-tubercular drugs to the infected lung tissue. Anti-tubercular drug loaded surfactants nanoparticles for inhalation therapy were proposed by Chimote et al. These nanoparticles act as anti-atelectatic agents and can stabilize the alveoli integrity by preventing alveolar collapse thereby allowing drug to reach the diseased alveoli uniformly in a non-invasive manner. Such nanoparticulate aerosols would be a significant improvement over existing therapies in pulmonary tuberculosis [93, 94].

Niosomes
Niosomes has similarity to that of liposomes and they are mainly composed of non-ionic surfactant and with or without incorporation of lipids. Jain and Vyas prepared micro-sized (8–15 μm) RIF-loaded niosomes. In vivo studies revealed that, depending upon the size of niosomes, up to 65% of the drug can be localized in the lungs [95]. Niosomes (1–2 μm) were prepared using sorbitan esters (Span® 20, 40, 60, 80 and 85) and cholesterol in a 50:50 percent mol fraction ratio [96]. They showed increase in entrapment efficiency with increase in hydrophobicity of the surfactant. In vivo biodistribution showed, higher RIF concentrations in thoracic lymph nodes via the intraperitoneal route (46.2% of the administered dose) for Niosomal formulations as compared to free drug (13.1%). These findings suggested that compartmentalization of the drug took place in the lymphous tissue. The same formulation when injected intravenously, about 7.3% of the drug was found in the thorax with the accumulation level lower than the 11.5% obtained by free RIF. In another study, Mullaicharam and Murthy studied the organ biodistribution of RIF niosomes (5 mg/mL) and drug solution following intravenous and intratracheal administration in albino rats. Niosomes mainly accumulated in the lung, liver and kidney with the organ to serum AUC ratios being 117,060 for lung/serum, 67 for liver/serum and 3068 for kidney/serum following iv administration. In contrast, administration of free RIF resulted in a less selective delivery (558.3 for lung/serum, 16.1 for liver/serum and 332.6 for kidney/ serum). After intratracheal administration, niosomes showed 145-fold increase as compared to free drug, representing targeting potential of developed carriers [97]. In another study, RIF loaded Niosomes were prepared by reverse phase evaporation method and charge was induced by using Di Cetyl Phosphate [98]. Attempts were also made to incorporate INH in Niosomes [99, 100].

Dendrimers
Dendrimers represent a novel class of structurally controlled three dimensional macromolecules that radiate from a central core and are mainly derived from a branches-upon-branches structural design. Dendrimers are well defined, highly branched macromolecules. Kumar et al. developed mannosylated fifth generation (5G) PPI dendrimeric nanoparticles for delivery of RIF to macrophages. Drug encapsulation mainly depend on hydrophobic interactions and hydrogen bonding contributing to the physical binding of the drug to the core. High haemolysis levels were observed for amine-terminated dendrimers. Mannose on surface significantly reduced the haemolytic toxicity of the nanocarriers from 15.6 to 2.8%. RIF-containing dendrimers reduced haemolytic effect of free RIF from 9.8 to 6.5%. A similar effect was observed in epithelial cell line of kidney when tested for viability; encapsulation significantly improved the survival of the cells from approx. 50% (free RIF) to 85% (RIF dendrimers). The phagocytic uptake of RIF and RIF-loaded dendrimers in alveolar macrophages harvested from rat lungs showed a clear increase
in the intracellular concentration of the antibiotic [101]. A more recent reports investigated 4G and 5G PEGylated-PPI dendrimers for sustain the delivery of RIF. PEGylation resulted in a significant increase in drug entrapment for 4\textsuperscript{th} and 5\textsuperscript{th} generation derivatives. PEG-grafted dendrimers showed low haemolytic activity (1–3\%) as composed the NH2-terminated ones (14–20\%) [102]. Researchers from Monash Institute of Pharmaceutical Science (Melbourne, Australia) developed PEGylated Polylysine dendrimers in collaboration with Starpharma Holdings Ltd for cancer, HIV, tuberculosis and lymphatic disease conditions.

**Cyclodextrin inclusion complexes**

Complexation approach with Cyclodextrins (CD) was applied for encapsulation of RIF by means of different CD molecules. Ferreira et al., prepared inclusion complexes of RIF with hydroxyl propyl β cyclodextrin (HPβCD) [103]. The complexation increased aqueous solubility of the drug linearly to the concentration of the CD used. The $^1$H and $^{15}$N NMR analysis of free and complexed RIF revealed important changes in pipеразине ring peaks of the drug, suggesting the interaction of this region with the hydrophobic core of HPβCD. Furthermore, UV analysis indicated an absence of strong bonding between the drug and the carrier [104].

Rao et al. compared complexation with β-CD and HPβCD of RIF in order to improve the chemical stability and aqueous solubility of the drug. Phase solubility studies showed a 1:1 molar ratio was apparent; the stability constants found to be 58.13 and 76.37 for the pristine and the modified CD, respectively indicating a stronger interaction between the drug and the HPβCD. IR spectroscopy further revealed that the interaction was through the пiperазине group of RIF. All the complexes improved the thermal stability of the drug. Antibacterial activity was assayed in M. tuberculosis for complexes showed a significant decrease in the MIC (minimum inhibitory concentration) from 64 to 32 μg/mL, this could be due to a better permeation of the drug through the wall of the bacilli [105]. In another study, poorly-water soluble nitroimidazole P-824 (a new anti-TB drug effect against drug-sensitive and multi-drug resistant bacilli) HP-γ-CD complex showed reduction in bacterial load in the lungs at 50 and 100 mg/kg doses [106]. To enhance the pulmonary bioavailability of poorly soluble drugs with improved biocompatibility and reduced toxic effect have been investigated for local delivery of drugs to the lung [107]).

**Nanotoxicological issues**

Nanoparticles interact with living cell at different levels depending upon the size. Particle size play very important role while discussing about nanotoxicity. Various studies suggest that nanoparticles less than 100 nm can enter cells, those with diameters < 40 nm can enter the cell nucleus and those that are <=35 nm can pass through the blood–brain barrier and enter the brain [108]. The knowledge about nanotoxicity help to identify the key safety issues in implementing nanoscience for health application, by investigating interaction of nanoparticles and biological systems (i.e., proteins, cells) and elucidating the relationship between the physical and chemical properties like size, shape, surface chemistry, composition, and aggregation with respect to toxic biological responses. Oxidative stress induced by nanoparticles can enhance inflammation through up regulation of redox-sensitive transcription. Nanoparticles can also alter mitochondrial function as well as cellular redox signaling. However, beside pharmacological activity, efficacy and pharmacokinetic studies it is very important to investigate toxicity effects of developed carrier systems at cellular level. Use of approved materials by official authority in development of carrier system would be helpful in minimizing toxic effects. International Alliance for Nano EHS Harmonization (IANH), working in direction of setting in vitro and in vivo nanomaterial Nano EHS testing protocols that are validated to produce the same results in multiple laboratories internationally and correlating the obtained results with obtained or predictive in-vivo data [109].

**New drug candidates in Clinical trials**

Currently available TB drug regimen is 40 years older comprising of administration of RIF and INH [110, 111]. The WHO recommends DOTS (directly observed treatment, short course) which consist of combination of isoniazid, rifampicin, pyrazinamide and ethambutol or streptomycin (World Health Organization, 2010). Noncompliance to therapy causes MDR (multi drug resistant) and extensively drug resistant (XDR) TB strains for which the treatment last for 2 years. Most of the currently available anti-TB drugs are ineffective toward latent tuberculosis infections except RIF and PZA [112-114]. This generates need of more effective treatment with
shorter duration and increased patient compliance. In this direction, various anti-TB drugs having novel mechanism of action have been synthesized and tested in-vitro. The American biolabs patented ASAP (silver) solutions for clinical treatment of various viral and bacterial diseases including TB [115, 116] and being successfully used in African hospitals to treat malaria, cholera, AIDS, flu, respiratory infections (pneumonia, bronchitis), tuberculosis, gonorrhea, MRSA (methicillin resistant staphylococcus aureus), hepatitis etc. Currently, fluoroquinolones moxifloxacin and gatifloxacin, the diarylquinoline are going under clinical trials [117, 118]. Clinical trials for Rifaxano - a combination of the four main first-line TB drugs are scheduled in 2012 and the drug should be possibly available in government clinics by 2016. These drugs are coated with nano-sized particles with chemicals which will enable them to stick to the intestinal wall and release the drug for longer time [119]. A patent has been filed for compound, OPC-67683 (nitrodihydroimidazo-oxazole) by Otsuka Pharmaceutical Co., Ltd. in 2003 and in 2006, Phase I clinical trial was conducted in Japan. Otsuka filed a PCT patent application covering 2,3-dihydro-6-nitroimidazo- (1,2-b) oxazole compounds for TB treatment [120, 121].

Recently Phase II trials were sucessfully completed for OPC-67683 [122]. PA-824 (prodrug with long half life, inhibit bacterial cell wall lipid and protein synthesis) showed high in-vitro activity against M. tuberculosis and has not shown cross resistant to currant anti-TB drugs [106, 123]. PA-824 is currently undergoing Phase II clinical trial (Global alliance for TB drug development, 2007). Whereas for SQ-109 (contains unsaturated isoprenyl units and adamantly ring) [124] completed Phase Ia trials in 2007 concluded that one-a week dosing may be achievable. Additional Phase Ib clinical study was performed to demonstrate safety of daily administration as single drug and as combination therapy to evaluate safety and efficacy in patients with pulmonary TB [124].

A diarylquinoline (DARQs) was being developed by Johnson & Johnson as R207910 and Tibotec as TM207 [125]. It has higher potency against drug susceptible, MDR and XRD tuberculosis strains and is 100 times more effective than conventional combination of RIF, INH and PYZ [126]. Johnson & Johnson found high bioavailability for TMC-07 oral solutions in clinical trials (Phase II). Hence studies on Solid dosage formulations are under development.

FASgen, Inc. is planning to conduct clinical trials on compound FAS 20013 (90% orally bioavailable) which is very efficient to eliminate > 99% of bacilli (additionally latent bacilli) within 24 h [127]. Nitroimidazopyran PA824 was developed by the Global Alliance for Tuberculosis Drug Development. Linezolid, an oxazolidinone drug used in short-term for various bacterial infections including TB (clinical trial.gov identifier NCT00727844) and for mitronidazole for treatment of pulmonary tuberculosis (clinical trial.gov identifier NCT00425113) is under phase II trial. The bicyclic nitroimidazole PA-824 induces the killing of both replicating and non-replicating M. tuberculosis is currently in Phase II clinical trials [128].

Other drug molecules are in various phases of preclinical and clinical trials and very little information is available. Lupin conducted multidose phase I clinical trial for a pyrrole derivative, LL-3858, an INH analogue (Sudoterb®) [129]. Another compound, OPC-37306 is active against drug susceptible and resistant M. tuberculosis strains and was discovered in screening studies of the inhibition of mycolic acid biosynthesis. SQ-609 acts by interfering in cell wall synthesis (Sequella Inc., 2007) while SQ641 inhibits enzyme translocase I which is required for synthesis of cell wall peptidoglycan [130]. Fluoroquinionolines, capuramycins (RS-118641), oxazolidinones (Linezolid), beta-sulfonylcarboxamides, pleuromutilin, erytromycin and various other semisynthetic derivates of natural product rifampicin viz. rifapentine, rifabutin, rifalazil and rifametane are currently in various phase trial [131].

**CONCLUSIONS**

Developments in nanoparticle-based delivery systems represent a cost-effective, practical and promising alternative for potential TB chemotherapy. Improved drug bioavailability and reduction of the dosing frequency, feasibility of the versatile routes of drug administration, long term stability may serve as basis for better management of the disease. Nanoparticles can be incorporated into various solid dosage forms like tablet, capsules, microparticles & granules would improve efficacy and practicability. The possibility of using drug carriers made from natural polymers (e.g., chitosan or alginate) represents an attractive perspective.
**Table 1:** Various tubercular chemotherapeutic studies in clinical trial and their sponsors.

<table>
<thead>
<tr>
<th>Aim of Study/drug candidate</th>
<th>Sponsor</th>
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<tbody>
<tr>
<td>Metronidazole for Pulmonary TB (South Korea)</td>
<td>National Institute of Allergy and Infectious Diseases (NIAID) NCT00425113</td>
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<tr>
<td>Pharmacokinetics and Pharmacodynamics of High Versus Standard Dose Rifampicin in Patients With Pulmonary TB (High RIF)</td>
<td>African Poverty Related Infection Oriented Research Initiative (NCT00760149)</td>
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<tr>
<td>TMC207-C202: Study to Evaluate Bactericidal Activity of Multiple Oral Doses of TMC207 in Subjects With Sputum-Smear Positive TB</td>
<td>Tibotec BVBA (NCT00523926)</td>
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<tr>
<td>Treatment of Latent TB Infection With Isoniazid</td>
<td>Instituto Nacional de Salud Publica, Mexico (NCT00293228)</td>
</tr>
<tr>
<td>A Randomized Clinical Trial Comparing 4RIF vs. 9INH for Treatment of Latent TB Infection (LTBI) - Effectiveness</td>
<td>McGill University (NCT00931736)</td>
</tr>
<tr>
<td>Tuberculosis Treatment Shortening Trial</td>
<td>National Institute of Allergy and Infectious Diseases (NIAID) NCT00130247</td>
</tr>
<tr>
<td>Linezolid to Treat Extensively-Drug Resistant TB</td>
<td>National Institute of Allergy and Infectious Diseases (NIAID) NCT00727844</td>
</tr>
<tr>
<td>TBTC Study 29: Rifapentine During Intensive Phase TB Treatment</td>
<td>Centers for Disease Control and Prevention NCT00694629</td>
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<tr>
<td>Rifapentine Plus Moxifloxacin for Treatment of Pulmonary TB</td>
<td>Johns Hopkins University NCT00728507</td>
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<tr>
<td>A Controlled Trial of a 4-Month Quinolone-Containing Regimen for the Treatment of Pulmonary TB</td>
<td>Institut de Recherche pour le Developpement (NCT00216385)</td>
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<tr>
<td>High-Dose Isoniazid Adjuvant Therapy for Multidrug Resistant Tuberculosis</td>
<td>GSVM Medical College NCT00513396</td>
</tr>
<tr>
<td>Pharmacokinetic Study for Anti-tuberculosis Drugs (TBPK)</td>
<td>Taipei Medical University WanFang Hospital (NCT00948077)</td>
</tr>
<tr>
<td>High Dose Rifapentine Pharmacokinetics, Tolerability and Safety Dosage Rifapentine for Treatment of TB (TBTC-29PK)</td>
<td>Centers for Disease Control and Prevention NCT01043575</td>
</tr>
<tr>
<td>PA-824-CL-007: Phase Ila Evaluation of Early Bactericidal Activity in Pulmonary TB</td>
<td>Global Alliance for TB Drug Development (NCT00567840)</td>
</tr>
<tr>
<td>TBTC Study 23: Treatment of HIV-Related TB Using a Rifabutin-Based Regimen</td>
<td>Centers for Disease Control and Prevention (NCT00023361)</td>
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<tr>
<td>TBTC Study 27/28 PK: Moxifloxacin Pharmacokinetics During TB Treatment</td>
<td>Centers for Disease Control and Prevention (NCT00164463)</td>
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<tr>
<td>Study of Daily Rifapentine for Pulmonary Tuberculosis</td>
<td>Johns Hopkins University (NCT00814671)</td>
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<tr>
<td>Randomized Trial for Pharmacogenomics-Based Tuberculosis Therapy (RT-PGTT)</td>
<td>Osaka University (NCT00298870)</td>
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</table>
Fig. 3: List of drugs (along with site of action) used in TB chemotherapy and patented new drugs

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