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Nanomedicine for tuberculosis: Insights from animal models

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ABSTRACT

Patient noncompliance to current tuberculosis (TB) therapy owing to multidrug administration daily leads to treatment failure and emergence of multidrug resistant and extensively drug resistant TB. To avoid the daily dosing, application of nanotechnology is the only viable solution by virtue of sustained release of drugs. Other potential advantages of the system include the possibility of selecting various routes of dosing, reduction in drug dosage/adverse effects/drug interactions, targeting drug resistant and latent bacteria etc. Plenty of work has been done in animal models of TB to support the notion that nanomedicine provides a ray of hope to encounter TB effectively; certain crucial questions are yet to be addressed.

Keywords: *Nanomedicine; Polymers; Drug delivery; Tuberculosis; Chemotherapy.*

INTRODUCTION

Science of drug designing and drug delivery has flourished also as an art with advances in knowledge of disease pathogenesis especially at the molecular level. A drug needs to be formulated in such a way so as to extract the maximum therapeutic benefit out of it. After oral administration drugs are degraded in gastrointestinal tract, by metabolic machinery, distributed in the systemic circulation and thereafter rapidly cleared from the body; all this results in lower concentrations of the drug for shorter duration at the target sites. To address these issues drugs are delivered by formulating them into drug delivery systems. When a drug formulation is designed in such a way that the rate and/or place of drug release are altered by virtue of drug delivery systems, the formulation is called a modified release system. The latter is also called as sustained/ controlled/ pulsatile/ slow/ extended/ prolonged release etc. Modified release is generally achieved by means of encapsulation technology which involves use of both natural and synthetic polymers [1] as drug delivery vehicles (Table 1).

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Moreover, non-polymeric drug carriers, e.g. lipids in the form of solid lipid nanoparticles (SLNs) are also gaining importance. Whatever be the carrier system, the ultimate aim of a drug delivery system is to improve the drug bioavailability by outfoxing one or several factors that negatively affect the bioavailability of drugs (Table 2).

Table 1. Natural and synthetic carriers used as drug delivery vehicles.

A. Natural carriers	B. Synthetic carriers
1. Proteins and polypeptides • Albumin • Fibrinogen/fibrin • Collagen • Gelatin • Casein	**1. Aliphatic polyesters and hydroxy acids** • Polylactic acid* • Polyglycolic acid • Poly(lactide-co-glycolide)* • Polyhydroxy butyric acid • Polycaprolactone
2. Polysaccharides • Alginic acid* • Starch • Dextrans/dextrin • Hyaluronic acid • Chitin • Chitosan*	**2. Polyanhydrides** **3. Polyorthoesters** **4. Polyalkylcyanoacrylate** **5. Polyamino acids** **6. Polyacrylamides** **7. Polyalkylcarbonates**
*Most commonly used polymer	

Table 2. Factors influencing drug bioavailability that can be improved by using an appropriate delivery system.

Parameters	Examples of drugs
Low shelf-life	Ethambutol
Unpalatability Extremes of pH Interaction with food Interaction with other drugs Poor solubility in intestinal fluid	Metronidazole Rifampicin in presence of isoniazid in acidic pH Rifampicin Rifampicin Danazol
Poor intestinal absorption	Streptomycin
Extensive first pass metabolism Subtherapeutic levels in plasma Short duration of stay	Propranolol Clotrimazole Azathioprine
Distribution to non-target organs	Anticancer drugs

DRUG DELIVERY SYSTEMS PROVIDE BETTER OPTION FOR MANAGEMENT OF TUBERCLULOSIS

The current multidrug regimen against tuberculosis (TB) needs daily administration for at least 6 months, patients often fail to adhere this complex regimen for such a long duration leading to patient noncompliance and treatment related side effects. That in turn leads to the development and spread of drug-resistant TB. Emergence of multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) raises serious concerns about the possibility of a future tuberculosis (TB) epidemic as limited therapeutic options are available. The situation becomes further worsening in the face of co-existing opportunistic infections such as human immunodeficiency virus (HIV). Moreover many countries lack the infrastructure to accurately diagnose MDR-TB and XDR-TB, thus intensifying the problem more. Although WHO initiated an ambitious TB control program referred to as directly observed treatment, short course (DOTS) wherein the administration of antitubercular drugs (ATDs) to a patient is directly supervised by a healthcare worker, its implementation especially in the rural areas is still a cherished desire. Obviously, if one could develop a system where it does not become necessary to administer ATDs on a regular basis, the strategy would certainly aid in improving patient compliance and avoid the drawbacks associated with the current chemotherapy. This is where the application of drug delivery systems by means of encapsulation technology (micro-encapsulation or nano-encapsulation) is likely to play its role by formulating ATDs into sustained release systems.

EVOLUTION OF ANTI-TUBERCULOSIS DRUG DELIVERY SYSTEM

Earlier, sustained release ATD formulation of isoniazid (INH) was attempted by incorporating it into three different polymers, viz. poly (methyl methacrylate), poly (vinyl chloride) and carbomer [2]. This was intended to achieve sustained plasma INH levels in fast acetylators of the drug. Using another polymer, Eudragit RS 100, the encapsulation and detailed release kinetics of INH were studied in the early 1990s [3]. Soon it was realized that aliphatic poly (esters) such as poly lactic acid (PLA), poly glycolic acid (PGA) and poly lactide-co-glycolide (PLG) (Figure 1) possess excellent biocompatibility, biodegradability and mechanical strength [4]. They can be used as drug delivery vehicles for a variety of macromolecules and are approved by the US Food and Drug Administration (FDA) for drug delivery. The era of aliphatic poly (esters) as ATD carriers began with the reporting of PLG as a carrier for INH [5]. The method involved dry mixing of INH and PLG to produce a film under pressure, which was implanted under the skin of mice and sustained plasma INH levels were maintained for 6 weeks. The levels obtained with the daily conventional free drugs for 6 weeks were comparable with the levels in the formulation-group just after only one dose. Significant antimycobacterial activity was seen in the liver/lung homogenates from the animals sacrificed 6 weeks post-implantation [5]. The method also worked well with other drugs, e.g. clofazimine (a second line ATD).

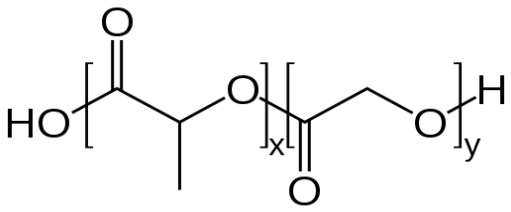

Fig.1. Structure of poly lactide-co-glycolide (PLG or PLGA).

The pharmacokinetic and pharmaco-dynamic studies revealed greater efficacy of PLG implants, incorporating isoniazid (INH) or pyrazinamide (PZA), in reducing colony forming unit (cfu) counts of M. tuberculosis in infected mice and rabbits. These results proved that indeed sustained release ATD delivery systems are possible to develop but the requirements of surgical intervention and partial anesthesia were major concerns.

This propelled scientists to go for injectable sustained release systems. Further in implants one of the most potent ATDs, rifampicin (RIF), was not evaluated. Accordingly three kinds of PLG-microparticles encapsulating RIF were prepared according to the emulsification and solvent

evaporation technique. They were called porous, nonporous (on the basis of their drug release behavior), or hardened [owing to use of high amounts of the hardening agent, poly (vinyl alcohol) (PVA)]. The latter proved to be the best formulation in terms of drug release because a single subcutaneous injection of hardened PLG microparticles exhibited sustained release of RIF in the organs (lungs, liver and spleen) of mice up to 6 weeks [6]. The slow but sustained release of RIF is due to its strong hydrophobic interactions with PLG. In order to evaluate application of the system for encapsulating hydrophilic molecules, INH was selected as the model drug. Following a single subcutaneous injection of PLG-encapsulated INH to mice, therapeutic drug levels could be maintained in the organs for 49 days as against 1 day in the case of free INH [7]. Encapsulation of INH in cellulose by multiple emulsion technique has also been reported but its in vivo evaluation has not been done so far [8]. Administration of single subcutaneous dose containing a combination of RIF and INH (encapsulated separately in PLG and mixed prior to dosing) resulted in maintenance of therapeutic drug levels for 6 weeks in mice. Moreover, the infected mice showed significant reduction of cfu counts in the organs after one injection of this formulation as compared to the controls [9-10].

In addition to their evaluation in parenteral route, PLG microspheres encapsulating ATDs have also been evaluated for oral route. For oral studies, PZA was also included and separate formulations were prepared for each drug followed by mixing. After administration of a single oral dose to mice, therapeutic drug levels in plasma were maintained for 3-5 days as compared to 1 day for free drug. With PLG as a vehicle for oral delivery of ATDs, improvements in most of the pharmacokinetic parameters were observed for each drug resulting in increased bioavailabilities of all drugs [11]. Free ATDs were cleared from body within 2 days while PLG encapsulated ATDs were detected in the tissues until 5-7 days. Accordingly dosing frequency of ATDs was reduced with oral PLG microparticles from once daily to once weekly [12]. This was shown by studies in which drug-loaded PLG microparticles were administered weekly for 6 weeks to M. tuberculosis infected mice. It resulted in a significant reduction in bacilli counts in the organs as did daily free drug therapy.

Thus reduction in dosing frequency was possible through the oral route. However several questions had to be addressed: would it be possible to achieve (i)a higher drug encapsulation efficiency, (ii) drug loading into PLG, (iii) improvement in drug bioavailability, and (iv) a further reduction in dosing frequency? Most of the answers were found in the concept of nanomedicine.

WHY NANOMEDICINE?

The role of nanoparticles in the development of drug delivery systems is well established [13-26] (Table 3). In comparison to microparticles nanoparticles achieve a higher drug encapsulation and loading that result in enhanced bioavailability of encapsulated drugs. They dissolve rapidly in the gastrointestinal tract which can increase drug uptake as the local concentration of drug may be higher than conventional dosage forms. Further, in contrast to microparticles, nanoparticles cross the intestinal permeability barrier directly via transcellular/paracellular pathways which help for better delivery of the encapsulated drugs into the circulation [13]. Several methods have been reported to obtain particles in the nano-range [27] (Table 4). In addition to oral route, nanoparticles can be administered by other routes as well.

Table.3. Important nanoparticle drug formulations. [Numbers in parentheses indicate the reference numbers].

Drug	Category
Heparin	Anticoagulant [13]
Betamethazone	Corticosteroid [14]
Budesonide	Corticosteroid [15]
Enalaprilat	Antihypertensive [16]
Bovine serum albumin	Protein [17]
Rolipram	Anti-inflammatory [18]
Praziquantel	Anti-helminthic [19]
Cyclosporine	Immunosuppressant [20]
Insulin	Hormone [21]
Octyl methoxy cinnamate	Sunscreen [22]
Digitoxin	Cardiac glycoside [23]
Itraconazole	Antifungal [24]
Clotrimazole, Econazole	Antifungal [25]
Moxifloxacin	Antibiotic [26]

Table 4. Various techniques employed for preparation of PLG-nanoparticles.

Techniques	Merits/demerits
Emulsion/evaporation	Poor entrapment of hydrophilic drugs
Double emulsion/evaporation	Good entrapment of hydrophilic/hydrophobic drugs
Salting out	Lengthy purification process
Emulsification-diffusion	Quick process
Solvent displacement/nanoprecipitation	Poor entrapment of hydrophilic drugs
Emulsification-diffusion-evaporation	Better reproducibility of size/shape of nanoparticles

Orally administered ATD-nanoparticles

Almost ten years back, three frontline ATDs, i.e. RIF, INH and PZA were co-encapsulated in PLG nanoparticles (PLG-NP) prepared by the double emulsion and solvent evaporation technique [28]. Particle size ranged from 186-290 nm with drug encapsulation efficiency of 60-70% for all three drugs. Table 5 summarizes all the variables that were found to influence the drug encapsulation efficiencies. The formulation was evaluated for its in vivo pharmacokinetic and pharmacodynamic potential at therapeutic doses, i.e. RIF 12 mg/kg + INH 10 mg/kg + PZA 25 mg/kg of body weight, or at sub-therapeutic doses [29]. Following a single oral administration of drug-loaded PLG-NP to mice, the plasma drug levels were maintained above their minimum inhibitory concentration (MIC90) for 6-9 days in the plasma, whereas no drug was detectable beyond 12 h following the oral administration of free drugs (alone or mixed with drug-free PLG-NP) [28]. At different time points the mice were sacrificed and the drugs were analyzed in homogenates of lungs, liver and spleen. The drugs were detected above MIC in organs for up to day 9. Therefore, based on the organ drug profile, the therapeutic schedule to be followed in infected animals comprised the formulation being administered every 10 days. Keeping in view dose dependent toxicity of ATDs, it was important to determine whether repeated administration of drug-loaded PLG-NP would result in any drug accumulation in the organs. Studies in which tissue drug levels were monitored on every 10th day following the repeated administration of the formulation showed no evidence of any drug accumulation in the organs tested, i.e. lungs, liver and spleen. The chemotherapeutic evaluation of free drugs administered daily (46 doses) and drug-loaded PLG-nanoparticles administered every 10 days (5 doses) orally to M. tuberculosis infected mice showed no detectable tubercle bacilli compared with a high bacterial load in lungs/spleen of untreated mice [28]. Interestingly, the results pertaining to the biodistribution, pharmacokinetics and chemotherapeutic efficacy of the formulation in mice were similar when carried out in a higher rodent, i.e. guinea pigs [29].

In humans ethambutol (EMB) is used during intensive phase of therapy because it hastens the rate of sputum conversion [30]. Accordingly PLG-NP encapsulating EMB were formulated and administered to mice, therapeutic drug concentrations were maintained in the plasma for 3 days and in organs up to day 7 [31]. PLG-NP formulation containing four drugs (ethambutol + three-drug regimen) after 4 weeks of therapy (once/10th day) resulted in no detectable cfu [31]. Thus, with the four-drug combination in PLG nanoparticles, it was possible to reduce the number of doses from 28 to 3. In the studies reviewed above, experimental infection had been established by the intravenous route. However, it was also important to evaluate the efficacy of the PLG-nanomedicine formulation in animals infected via the aerosol route because the latter is the natural mode of acquiring TB. In guinea pigs infected via the aerosol route, 5 oral doses of ATD-loaded PLG-NP and 46 doses of free drugs still proved to be equi-efficacious [32]. This further strengthened the concept of ATD-nanomedicine. Encouraging results have been obtained with ATD (particularly RIF) loaded in other synthetic polymer-based nanoparticulate systems such as poly butylcyanoacrylate [33] and poly ε-caprolactone [34].

Table.5. The important variables influencing the encapsulation of ATDs in PLG- nanoparticles prepared by the double emulsion/evaporation technique.

Variable	Drug encapsulation efficiency (%)
1. Drug:polymer	
1:0.5	40-48
1:0.8	44-56
1:1	60-70
1:1.5	60-70
1:2	60-70
2. Concentration of polyvinyl alcohol (%w/v)	
0.5	54-55
1.0	60-70
1.5	60-70
2.0	54-66
3. Polyvinyl alcohol:dichloromethane (v/v)	
100:10	40-50
50:10	55-65
10:10	60-70
8:10	60-70
5:10	45-65

- **Surface functionalized orally administered ATD-nanoparticles**

Adhesion of a drug carrier to a mucosal surface is limited by the turnover time of the mucus gel layer, which are only a few hours for most mucosal surfaces. To circumvent this problem, polymeric drug carriers can be attached to certain cytoadhesive ligands that bind to epithelial surfaces through specific receptor mediated interactions. Lectins, a structurally diverse class of proteins resistant to proteolytic degradation which are found in organisms ranging from viruses and plants to humans, are appropriate candidates for this approach [35]. Wheat germ agglutinin (WGA), one of the least immunogenic lectins, has its receptors on intestinal as well as alveolar epithelium, thus potentiating its use for oral as well as aerosol drug delivery [36]. The sustained release profile of all the ATDs was improved as the drugs were detectable in tissues until day 15 in the case of the lectin-based ATD-nanomedicine against day 10 in the case of the uncoated formulation [37]. In M. tuberculosis infected guinea pigs, three oral doses of ATD-loaded lectin PLG-NP (spaced 15 days apart) resulted in undetectable cfu against 46 conventional doses of oral free drugs.

ATD-nanoparticles administered via pulmonary route

Respiratory route represents a novel means of delivering ATDs directly to the lungs. Inhalable nanoparticles have better mucosal cell adherence, hence enhancing net drug delivery to the lungs [38]. In addition, nanoparticles are efficiently taken up by phagocytic cells (such as alveolar macrophages, the abode of M. tuberculosis), and subsequently release their payload [39]. Upon aerosolization, inhalable PLG-NP co-encapsulating RIF, INH and PZA, were found to possess a mass

median aerodynamic diameter (MMAD, determined on a 7-stage Andersen Cascade Impactor) of 1.88 μm, suitable for deep lung delivery. A single nebulization of the formulation to guinea pigs was able to maintain therapeutic drug concentration in the plasma for 6-9 days and in the lungs for 9-11days. Nebulized drugs showed dramatic improvement in the half-life, mean residence time and bioavailability, it is worth to mention that free drugs were not nebulizable. In M. tuberculosis infected guinea pigs, 5 nebulized doses of the nanomedicine spaced 10 days apart resulted in undetectable cfu in the lungs replacing 46 oral doses. This was the first report of an inhalable TB-nanomedicine [40]. By this method it was possible to co-administer multiple ATDs encapsulated in nanoparticles with a better therapeutic response.

Other workers have reported that RIF formulated into spray-dried PLG-NP and administered intra-tracheally to guinea pigs could maintain pulmonary drug levels for 8 h in contrast to free RIF. Systemic levels were also attained with the nanoparticles for 6-8 h [41]. Ohashi et al. [42] employed yet another interesting approach wherein PLG-NP encapsulated RIF were formulated into mannitol microspheres. Following inhalation, efficient uptake of these microspheres by alveolar macrophages was demonstrated in rats in contrast to low uptake of simple RIF-PLG microspheres. Based on an in vivo imaging study, the authors concluded that the nanoparticles were cleared slowly than the microparticles, resulting in their pulmonary retention.

Since lectin receptors are widely distributed in the respiratory tract, it was worthwhile to assess the chemotherapeutic potential of lectin-functionalized PLG-NP. Upon nebulization to guinea pigs, therapeutic drug concentrations were maintained in the plasma for 6-10 days and in the organs for 15 days. A series of experiments have proved that 46 conventional doses could be reduced to 5 nebulized doses of PLG-nanomedicine and, further, to just 3 doses with lectin-PLG-NP.

Another process called insufflation provides an alternative to deliver ATDs directly to the lungs. This includes spray drying of drug-loaded particles to form inhalable powders followed by direct pulmonary deposition. Excellent work has been carried out in this area by research groups such as those of Hickey and Edwards. A promising anti-TB candidate molecule, PA-824 (a nitroimidazopyran), was formulated into dry powder porous particles stable at room temperature for 6 months and under refrigerated conditions for at least 1 year [43]. Insufflation to guinea pigs resulted in sustained drug levels in the lungs upto 32 h which was significantly higher than oral PA-824. The lungs and spleens of M. tuberculosis infected guinea pigs receiving high doses of aerosolized PA-824 exhibited a lower cfu and tissue damage compared to the untreated animals [44]. The procedure was also suited for lower animals such as mice (where wet nebulization to an individual animal is difficult) and could be applied to other ATDs like capreomycin as well as vaccines [45-47]. The technology is a stepping stone towards pulmonary delivery of ATDs in a quick (less time required compared with nebulization) manner.

Injectable ATD-nanoparticles

The bioavailability of injectable drug is absolute i.e. 100% of the administered drug is instantly available to the systemic circulation. The subcutaneous and intramuscular routes also provide bioavailability profiles close to the intravenous route. In TB-infected mice, subcutaneous administration of a single injection of drug-loaded PLG-NP resulted in sustained drug levels in the plasma for 32 days and in the organs for 36 days. There was a complete bacterial clearance from the organs, demonstrating a better efficacy compared with daily oral free drug treatment [48]. Although the microparticulate system also demonstrated a significant reduction in bacterial load, it failed to result in complete tissue sterilization [9-10]. Other advantages of the nanoparticulate system over the microparticulate one are enlisted in Table 6.

Recently, mannose-conjugated gelatin nanoparticles (260-380 nm) encapsulating INH were prepared by a two-step desolvation method. Upon intravenous administration, the formulation achieved lung targeting in TB-infected mice leading to a significant reduction in cfu, besides minimal hepatotoxicity [49].

Table 6. PLG-nanoparticle ATD carriers versus PLG-microparticle ATD carriers.

Parameters	PLG nanoparticles	PLG microparticles
Particle size	186-290 nm	10-12 μm
Type of formulation	Single formulation encapsulating multiple drugs	Separate formulations for each drug
Drug encapsulation (%)	55-73%	8-40%
Drug:polymer	1:1 for each drug	Varies with the drug
Polymer consumption	Low	High
Sustained drug release in plasma following oral administration to experimental animals	6-9 days	3-4 days
Feasibility of nebulization	Yes	No
Increase in drug bioavailability	9-53 fold	7-9 fold
Schedule of oral therapy in experimental TB models	Every 10-15 days	Weekly
Therapeutic benefit	Complete	Partial

ALGINATE-BASED ATD-NANOPARTICLES

US FDA has already approved alginic acid, a natural co-polymer of guluronic acid and mannuronic acid (Figure 2), for oral usage. At present, the polymer is employed clinically for the supportive treatment for reflux esophagitis. Besides, it is used as a binding and disintegrating agent in tablets, a suspending and thickening agent in water-miscible gels/lotions/creams, and a stabilizer for emulsions.

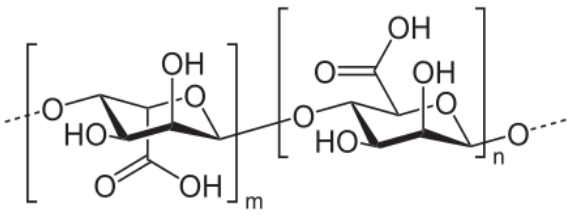

Fig.2. Structure of alginic acid.

There are several advantages of using alginate as a drug delivery vehicle (Table 7). These include a relatively high aqueous environment within the matrix, adhesive interactions with intestinal epithelium, a mild room-temperature drug(s) encapsulation process free of organic solvents, a high gel porosity allowing high diffusion rates of macromolecules, the ability to control this porosity with simple coating procedures using polycations such as chitosan or poly (L-lysine) (PLL), and dissolution/biodegradation of the system under normal physiological conditions [50-52]. Owing to these properties, alginate has been used as a carrier for the controlled release of numerous molecules of clinical interest, e.g. indomethacin [53], sodium diclofenac [54], nicardipine [55], dicoumarol [56], gentamicin [57], vitamin C [58], ketoconazole [58], amoxycillin [59], insulin [60], anticancer drugs [61] and ATDs [62-64]. Alginate-based drug delivery systems are broadly divided into two types ; the membrane-reservoir system and the matrix system. In the membrane-reservoir system, the drug release from the inner reservoir core is controlled by the polymeric encapsulating membrane having a specific permeability. Increase in the thickness of the coat/membrane decreases the release rate. Moreover, the co-encapsulation of certain nonpolar substances may further reduce the release rates. This property was advantageously used in the encapsulation and controlled release of indomethacin, where the sudden release of the drug is highly undesirable because indomethacin is an

irritant to the gastrointestinal mucosa [53]. On the other hand, in the matrix system or more specifically the swelling-dissolution-erosion system, the drug molecules are dispersed in a rate controlling polymer matrix. The matrix swelling as well as dissolution/erosion occurring concomitantly at the matrix periphery is the factors that modulate drug release [65]. Broadly speaking, factors related to the development of a particular formulation as well as factors encountered once the formulation is inside a living system can influence alginate-based drug delivery systems to a great extent (Table 8).

Alginate exhibits satisfactory hemocompatibility and its hydrophilic nature prevents its rapid clearance by the mononuclear phagocyte system on intravenous administration. This imparts a long circulation half-life to alginate nanoparticles. Unlike alginate, the more traditional neutral polymers or liposomes require additional incorporation of hydrophilic co-polymers or polyethylene glycol fatty acid derivatives to enhance their hydrophilicity. Alginate nanoparticles have been prepared and stabilized with PLL/chitosan, encapsulating ATDs (RIF, INH, PZA and EMB). The drug: polymer ratio was kept at 7.5: 1 which is better compared with PLG-NP, where the ratio was 1: 1. Thus, the alginate formulation allows more loading of the drug with lower consumption of the polymer. The formulation provides a sustained release for 7-11days in plasma and 15 days in the organs following a single oral dose [66]. In M. tuberculosis infected mice, 3 oral doses of the formulation administered fortnightly led to complete bacterial clearance from the organs replacing 45 doses of free drugs [67]. Comparable results were obtained in guinea pigs by the oral [68] as well as the respiratory route [69]. Therefore, alginate nanoparticles can certainly be considered a better ATD-nanomedicine over PLG-NP [70]. Encouraging results have been obtained with ATD (particularly RIF) loaded in other natural carrier-based nanoparticulate systems. For example, Saraogi et al. [71] prepared RIF-encapsulated gelatin nanoparticles by a two-step desolvation process resulting in 264±11.2 nm sized particles. In contrast to free drug, the RIF-gelatin exhibited significantly improved pharmacokinetics as well as pharmacodynamics in TB-infected mice.

Table 7. Advantages of using alginate as a drug delivery vehicle.

- A natural polymer
- Large-scale production economically
- Compatible with a variety of substances
- Simple drug encapsulation process
- Mucoadhesive
- Biodegradable
- Non-toxic
- Formulation of different delivery systems
- Sustained drug release
- Enhanced drug bioavailability
- Applications in biotechnology

Table 8. Factors influencing drug encapsulation/release from alginate-based systems.

- pH of the surrounding medium
- Relative proportion of G and M residues
- Molecular weight and viscosity of alginate
- Drug-polymer ratio
- Ionic nature of the drug
- Nature and amount of cross-linker
- Gelling time
- Variation in particle size
- Addition of regulatory molecules

SOLID LIPID NANOPARTICLE-BASED ADT-DELIVERY

Solid lipid nanoparticles (SLNs) are nanocrystalline suspensions in water, prepared from lipids which are solid at room temperature [72]. The SLNs are a new form of nanoparticulate carriers in addition to the more conventional ones such as liposomes, lipid emulsions, and polymeric nanoparticles. The SLNs possess good tolerability (due to their derivation from physiological lipids), scaling-up feasibility, the ability to incorporate hydrophobic/hydrophilic drugs, and an enhanced stability of incorporated drugs. Thus, SLNs are unique in the sense that they combine the virtues of traditional nanoparticles while eliminating some of

their problems [73]. SLNs have been researched for formulation development and for the incorporation of drugs to improve their bioavailability as well as for targeted drug delivery [74-77].

SLNs can be prepared by precipitation in oil-in-water emulsions. The lipophilic core material of SLNs is dissolved in water-immiscible organic solvent that is emulsified in an aqueous phase. Upon evaporation of the solvent, the lipid precipitates and forms solid nanoparticles. SLNs can also be prepared from warm oil in water micro emulsions by mixing melted fatty acids or triglycerides with water, surfactant, and co-surfactant to form a transparent micro emulsion which is quenched and dispersed in cold aqueous medium. The lipid solidifies and forms the nanoparticles. A more recent and popular method is the preparation of SLNs by high-pressure homogenization [72].

The ATD-loaded SLNs have been prepared by the emulsion solvent diffusion technique to co-incorporate RIF, INH and PZA. The chemotherapeutic potential of the formulation was evaluated via the respiratory route in guinea pigs. It was observed that a sustained drug release was maintained for 5 days in plasma and for 7 days in the organs. Seven weekly doses of the formulation resulted in undetectable bacilli in the organs of TB-infected guinea pigs, replacing 46 conventional doses [78]. The possibility of administering this ATD-nanomedicine was also evaluated via the oral route and similar encouraging results were obtained [79].

ATD-NANOMEDICINE FOR OTHER FORMS OF TB: Cerebral, Drug-resistant and Latent

Owing to the increased mortality associated with Cerebral TB, it was worth exploring whether ATD-PLG-NP could achieve brain ATD localization. This concept was further strengthened by the fact that following oral administration, nanoparticles are distributed to extrapulmonary sites [28-29]. Indeed, a single oral dose of ATD-nanomedicine could attain drug levels in the brain for 9 days. In a murine TB model, 5 oral doses of the formulation administered every 10th day, resulted in undetectable bacilli in the meninges, as assessed on the basis of cfu and histopathology [80]. These results certainly merit evaluation in a higher animal model.

Keeping in mind the rising incidence of MDR-TB and its deadly alliance with HIV [81-82], the concept of nanomedicine was taken further to encapsulate second line ATDs as well. Lopes et al. [83] reported the nano-encapsulation of ethionamide and characterized the formulation but in vivo results have not been reported. Ten years later, ethionamide-loaded PLG-NP were prepared and evaluated by a different research group [84]. A single oral dose of the formulation produced sustained release of ethionamide for 6 days in plasma whereas the free drug was cleared in 6 h in mice. Ethionamide was detected in the organs for 5-7 days, suggesting a weekly therapeutic regimen in drug-resistant TB.

A more exciting development has been the nano-encapsulation of azole antifungals (clotrimazole, econazole) [85] and fluoroquinolones (moxifloxacin) [86] which have potent anti-mycobacterial activity against drug-sensitive [87- 88] and resistant [89] strains of M. tuberculosis as well as latent bacilli [90]. Kisich et al. [86] demonstrated that moxifloxacin encapsulated in poly (butyl cyanoacrylate) nanoparticles accumulated in alveolar macrophages 3 times more efficiently than the free moxifloxacin. Further, the encapsulated drug was detected intracellularly for 6 times longer duration than free drug even at similar extracellular levels. An intracellular concentration of 0.1μg/ml with encapsulated moxifloxacin was comparable to 1.0 μg/ml of free drug in terms of inhibiting mycobacterial growth [86]. In M. tuberculosis infected mice, 8 weekly doses of PLG-NP encapsulated triple-drug combination (moxifloxacin + econazole + rifampicin) resulted in complete bacterial clearance from the organs [26]. Moreover, the addition of moxifloxacin has the potential to shorten the duration of treatment [91]. Studies with econazole encapsulated in alginate nanoparticles demonstrated that the system has an edge over the PLG-NP, both in terms of pharmacokinetics as well as pharmacodynamics, reducing the dosing frequency by 15-fold [26, 85]. The system is worth exploring for intermittent therapy against MDR-TB and latent TB.

Streptomycin, one of the most cost-effective ATDs, is recommended in certain special cases, e.g. relapse/treatment failure, withdrawal of

INH and RIF, TB meningitis, co-treatment with HIV protease inhibitors and certain cases of MDR-TB [93]. However, it needs parenteral administration and has lower margins of safety due to its potential for nephrotoxicity and ototoxicity. To overcome these drawbacks, Streptomycin has also been formulated into an oral dosage form by nano-encapsulation which when administered orally to mice exhibited an enhanced bioavailability, therapeutic efficacy and no nephrotoxicity in contrast to free drug [92]. Reports of nanomedicine prepared with new anti-TB drugs (e.g. isoxyl) [94] or modified existing drugs (e.g. fullerene-INH conjugate) [95] have also appeared.

TOXICOLOGICAL ASSESSMENT OF ADT-NANOMEDICINE

ATD-loaded PLG-NP is a distinct new chemical entity (NCE) that merits careful toxicological assessment, both short-term (single dose or acute toxicity to determine the median lethal dose, LD50, i.e. the single dose that would produce mortality in 50% of the animals within 14 days) and long-term (multiple dose, i.e. subacute toxicity for 28 days and chronic toxicity for 90 days).

Assessment of single dose acute toxicity in mice revealed that the free drugs were toxic at 80 times the therapeutic dose and above whereas the PLG formulation was non-toxic even at 150 times the therapeutic dose (it was technically not possible to administer further higher doses to mice by gavage owing to the bulk of the formulation). Similarly subacute and chronic toxicity studies wherein the PLG formulation was administered on every 10th day showed no adverse effects with either drug-loaded or drug-free nanoparticles in mice or rats. Further the histopathological examination excluded the possibility of any minor changes in the organs [96]. Thus when applied to medicine, polymeric nanoparticles are less toxic [97- 98]. This should encourage researchers to further explore the safety profile of PLG that may expedite its approval for oral usage, besides undertaking toxicological evaluation in higher animal models as a prelude to clinical studies.

ATD-NANOMEDICINE: ISSUES AHEAD

Although ATD-nanomedicine is a promising alternative to the current practice of TB-chemotherapy, there are various factors which impede its reach to human trials (Table 9). A potential problem in the large-scale production of synthetic polymer-based ATD-nanomedicine is the removal of residual organic solvents (e.g. dichloromethane used in preparing PLG-NP). Although the problem could be solved by temperature-controlled vacuum drying, the process is likely to increase the cost of production. A natural polymer-based delivery system such as alginate nanoparticles is a suitable alternative. Many crucial questions are yet to be addressed in animal models to mimic the human scenario of the disease and treatment in order to move towards clinical trials.

In order to ensure an optimum batch-to-batch drug loading into the nanoparticles, there is a need to adopt rigorous quality control measures. PLG requires storage under cold conditions (4-8OC) and as such there is a need to perform long term stability studies to assess the shelf-life of the formulation(s), keeping in view the bulk requirement of ATDs in rural areas of Asia and Africa with suboptimal drug storage conditions. Moreover, despite the evaluation of sustained release kinetics of various dosage forms of ATD-nanomedicine in animal models (Table 10), the exact dosing schedule to be followed in humans would be revealed only after the results of clinical studies become available.

Another point to be considered is the selection of the route of administration. Intermittent oral therapy would be more patient-friendly whereas treatment with inhalable or injectable ATD-nanomedicine would require supervision and medical expertise, at least during the initial few doses in adults and each time in children. Challenges to pulmonary delivery of ATDs including the issue of nebulization and insufflation have been discussed earlier. Lastly, it is emphasized that the clinical success of ATD-nanomedicine, its marketing and post-marketing surveillance would not only require firm governmental commitment but also a keen interest from leading pharmaceutical companies even if it means a less profitable business.

Table 9. Factors impeding ATD-nanomedicine from reaching human trials.

- Removal of residual organic solvents
- Cost of polymer/drug carrier
- Large-scale optimization of batch-to-batch drug loading
- Long-term stability studies
- Safety/toxicity profile of new chemical entities
- Efficient pulmonary delivery of a suitable dosage form (nebulization vs. insufflation)

Table 10. Evaluation of TB-nanomedicine in animal models.

Mode of delivery and dosage forms	Drugs evaluated	Animal model	Sustained release (days)		References
			Plasma	Organs	
Oral					
PLG-NP	RIF, INH, PZA	Mice, guinea pigs	6-8	9-11	28, 29
	EMB	Mice	3	7	31
	Streptomycin	Mice	4	7	92
	Ethionamide	Mice	6	5-7	84
	Econazole	Mice	5	6	25, 26
	Moxifloxacin	Mice	4	6	26
Lectin-PLG-NP	RIF, INH, PZA	Guinea pgs	6-14	15	37
Alginate-NP	RIF, INH, PZA, EMB	Mice, guinea pigs	7-11	15	66-68
	Econazole	Mice	6	8	25, 85
SLNs	RIF, INH, PZA	Mice	8	10	79
Inhalable					
PLG-NP, nebulized	RIF, INH, PZA	Guinea pigs	6-8	9-11	40
PLG-NP, insufflated	RIF	Guinea pigs	0.25	0.3	41
Lectin-PLG-NP	RIF, INH, PZA	Guinea pigs	6-14	15	37
Alginate-NP	RIF, INH, PZA, EMB	Guinea pigs	7-11	15	69
SLNs	RIF, INH, PZA	Guinea pigs	5	7	78
Injectable					
PLG-NP	RIF, INH, PZA	Mice	32	36	48

CONCLUSION

With the exception of quinolones and rifamycins, no significant additions have been made to the ATDs. The major drawbacks associated with new drug development include- an input of immense research efforts, cost and time; difficulty in targeting MDR and latent bacilli; and uncertainty with respect to toxicity and resistance [99]. These limitations act as the driving force behind the search for alternative therapeutic strategies. By employing nanotechnology, synthetic/natural polymer-based controlled release ATD-nanomedicine formulations have been developed, encapsulating first line as well as second line ATDs [100]. Besides offering the flexibility of selecting three different routes of administration, the PLG-NP exhibits a sustained drug release which allows replacement of daily conventional free drug treatment with intermittent doses of nanoparticle-based drugs [100]. In addition, the duration of chemotherapy can be reduced, drug bioavailability can be enhanced, and therapeutic efficacy can be maintained even at sub-therapeutic doses of the formulation. All these factors are critical in substantially curtailing the cost of treatment, reducing interactions with anti-HIV drugs, and better management of drug-resistant/ latent TB. Alginate nanoparticles are even more advantageous over PLG-NP in terms of simplicity of production, consumption of polymer, drug encapsulation/loading, and sustained release of drugs [100]. Therefore future research will focus more and more on this versatile biopolymer although exploration of other carriers will also continue. Nevertheless, aside from the issue of the choice of polymer and some key milestones yet to be achieved, nanomedicine may be the long sought solution for improving patient compliance in TB chemotherapy.

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