The Potential Advantages of Nanoparticle Drug Delivery Systems in Chemotherapy of Tuberculosis

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Nanoparticle-based drug delivery systems have considerable potential for treatment of tuberculosis (TB). The important technological advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation. Nanoparticles can also be designed to allow controlled (sustained) drug release from the matrix. These properties of nanoparticles enable improvement of drug bioavailability and reduction of the dosing frequency, and may resolve the problem of nonadherence to prescribed therapy, which is one of the major obstacles in the control of TB epidemics. This article highlights some of the issues of nanotechnology relevant to the anti-TB drugs.

Keywords: antituberculosis drugs; nanoparticles; tuberculosis therapy

Approximately one-third of the world population is infected with *Mycobacterium tuberculosis*, resulting in more than eight million new cases and two million deaths annually. Although potentially curative treatments have been available for almost half a century, tuberculosis (TB) remains the leading cause of preventable deaths in the world today. Recent implementation of the World Health Organization's strategy (directly observed therapy, short-course) has been problematic, and TB remains a major burden in many developing countries. One of the major problems is noncompliance to prescribed regimens, primarily because treatment of TB involves continuous, frequent multiple-drug dosing. Adherence to treatment and the outcome of therapy could be improved with the introduction of long-duration drug formulations releasing the antimicrobial agents in a slow and sustained manner, which would allow reduction in frequency and dosing numbers.

One way to solve this problem is the development of colloidal drug delivery systems. Liposomes are a well-known example of this strategy (1, 2). Other drug carriers (such as nanoparticles) represent an attractive alternative to liposomes. Today, versatility of particulate technologies enables tailoring of the nanoparticle-based drug delivery systems with consideration of the target, desired pharmacokinetic profile, and route of administration, The achievements and challenges of drug delivery using nanoparticles have been covered in numerous publications over the last few years. The goal of the present review is to highlight the potential advantages of this research strategy relevant to the treatment of TB. Application of nanoparticles for the development of vaccines is beyond the scope of this article. Preliminary data by this group of authors on nanoparticles for TB drugs were published in 2000 (24).

DEFINITION AND BACKGROUND

Nanoparticles for the purpose of drug delivery are defined as submicron ($< 1\mu m$) colloidal particles. This definition includes 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 (3). Alternatively, the drug can be covalently attached to the surface or 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, polyalkylcyanoacrylates), or solid lipids. In the body, the drug loaded in nanoparticles is usually released from the matrix by diffusion, swelling, erosion, or degradation.

The following are among the important technological advantages of nanoparticles as drug carriers: 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 designed to enable controlled (sustained) drug release from the matrix. The methods for nanoparticle preparation and characterization were addressed in numerous reviews, some of which are referenced here (4–8). Table 1 summarizes major data on nanoparticulate formulations of the anti-TB drugs.

ORAL ADMINISTRATION OF NANOPARTICLE-BASED TB DRUGS

Stability of nanoparticles offers the possibility of oral administration. The fate of nanoparticles in the gastrointestinal tract has been investigated in a number of studies (9–11). In general, the uptake of nanoparticles occurs as follows: (*1*) by transcytosis via M cells, (*2*) by intracellular uptake and transport via the epithelial cells lining the intestinal mucosa, (*3*) by uptake via Peyer's patches.

Pandey and colleagues (12) demonstrated that the nanoparticles provided sustained release of the anti-TB drugs and considerably enhanced their efficacy after oral administration. Three frontline drugs, rifampin (RMP), isoniazid (INH), and pyrazinamide (PZA) were coencapsulated in poly(lactide-co-glycolide) (PLG) nanoparticles. After a single oral administration of this formulation to

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TABLE 1. DRUG RELEASE AND THERAPEUTIC EFFICACY OF THE NANOPARTICLE-BASED FORMULATIONS OF THE FIRST-LINE ANTITUBERCULOUS DRUGS—RIFAMPIN, ISONIAZID, AND PYRAZINAMIDE

Delivery System	Animal Model	Administration Route	Duration of Drug Release (*d*)		Regimen Producing Sterilizing Effect in Lungs and Spleen	Ref
			Plasma	Organs		
PLG nanoparticles	Mice	Oral	6–9	9–11	5 doses every 10 d	12
	Mice	Subcutaneous	32	36	Single injection	30
	Guinea pigs	Aerosol	4–9	up to 10 d (each drug)	5 doses every 10 d	17
	Guinea pigs	Oral	4–9	up to 10 d (each drug)	5 doses every 10 d	17
Lectin-functionalized PLG nanoparticles	Guinea pigs	Oral	7–13	up to 15 d (each drug)	3 doses fortnightly	17
	Guinea pigs	Aerosol	6–14	up to 15 d (each drug)	3 doses fortnightly	17
Solid lipid nanoparticles	Guinea pigs	Aerosol	5	7	7 doses weekly	22

Definition of abbreviations: PLG = poly(lactide-co-glycolide); Ref = reference.
The drug-to-polymer ratio is 1:1 for each drug.

mice, the drugs could be detected in the circulation for 4 d (RMP) and 9 d (INH and PZA); therapeutic concentrations in the tissues were maintained for 9 to 11 d. In contrast, free (unbound) drugs were cleared from the plasma within 12 to 24 h after administration. Treatment of *M. tuberculosis*–infected mice with the nanoparticle-bound drugs (five oral doses every 10th day) resulted in complete bacterial clearance from the organs. Free drugs were able to produce bacterial clearance only after daily administration of 46 doses. Similar efficacy of the nanoparticle-bound drugs was also observed in guinea pigs (13).

At the same time, incorporation in microparticles was less effective: their drug-loading capacity was lower as well as the plasma half-life of the bound drugs (14, 15).

The behavior of polymeric nanoparticles in the gastrointestinal tract is influenced by their bioadhesive properties; adhesion of nanoparticles to the mucosa enhances the absorption of the associated drug, thus increasing its bioavailability. Thus, lectins have been shown to improve mucoadhesion of the drug due to the biorecognition of the lectin-grafted carriers by glycosylated structures in the intestine (16).

Accordingly, the efficacy of PLG-based formulations of anti-TB drugs were further improved by covalent attachment of wheat germ agglutinin (17). Oral administration of wheat germ agglutinin–coated PLG nanoparticles loaded with RIF, INH, and PZA in mice produced considerably extended serum half-life: detectable RIF serum levels were observed for 6 to 7 d and INH and PZA for 13 to 14 d (vs. 4–6 d and 8–9 d for nonmodified nanoparticles). All three drugs were present in lungs, liver, and spleen for 15 d. The lectin-modified formulations produced bacterial clearance in these organs after three oral doses administered every 14 d (vs. 45 daily doses of free drugs). As suggested by the authors, the prolonged circulation of drugs encapsulated in wheat germ agglutinin–grafted nanoparticles might be attributed to the fact that lectins enhance prolonged adhesion of the particles to the intestinal surface to allow (*1*) an increase in the time interval available for absorption and (*2*) a localized increase in the concentration gradient between luminal and serosal sides of the membrane.

POTENTIAL FOR THE INHALATION FORM APPLICATION

The potential advantages of direct delivery of the TB drug to the lungs include the possibility of reduced systemic toxicity, as well as achieving higher drug concentration at the main site of infection. Moreover, in contrast to the oral route of administration, inhaled drugs are not subjected to first-pass metabolism. A possible obstacle to using nanocarriers for pulmonary delivery is that their mass median aerodynamic diameter, an essential parameter for the particle deposition in the lungs, is often too small.

Nevertheless, the effectiveness of pulmonary drug delivery using nanoparticles was demonstrated in a number of studies (18). The pharmacokinetics and antibacterial effect of the nanoparticle-bound anti-TB drugs administered via respiratory route was investigated in guinea pigs (19). The dose was delivered via a suitable facemask connected to the compressor–nebulizer system. A single nebulization of RMP, INH, and PZA coencapsulated in PLG nanoparticles to guinea pigs resulted in sustained therapeutic drug levels in the plasma for 6 to 8 d and in the lungs for up to 11 d. This effect was similar to that obtained after oral administration of the nanoparticulate formulation of the same drugs. In nebulization of nanoparticles to *M. tuberculosis*–infected guinea pigs at every 10th day, no tubercle bacilli could be detected in the lung after only five doses of treatment, whereas 46 daily doses of orally administered drug were required to obtain an equivalent therapeutic benefit.

Administration to infected guinea pigs of nebulized RMP, INH, and PZA coencapsulated in wheat germ agglutinin–functionalized PLG nanoparticles was even more effective: three doses administered fortnightly for 45 d were sufficient to produce a sterilizing effect in lungs and spleen (17).

A sterilizing effect was also achieved when the drugs were loaded in solid lipid nanoparticles (20). 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. 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 (10).

INTRAVENOUS ADMINISTRATION

In contrast to microparticles with a diameter of more than 1 μm that cannot be administered via intravascular routes, nanoparticles are small enough to allow intracapillary passage followed by an efficient cellular uptake. When administered intravenously, the nanoparticles follow the route of other foreign particulates, including intracellular pathogens. They are endocytosed by resident macrophages of the mononuclear phagocyte system and by circulating monocytes. On the other hand, in the case of infections caused by intracellularly persisting microbes (e.g., *Brucella*, *Salmonella, Listeria, Mycobacterium*), macrophages become reservoirs for pathogens, thus representing one of the targets for delivery of antimicrobial agents.

Preferential uptake of nanoparticles by macrophages (mainly by Kupffer cells in the liver) is achieved by the physicochemical properties of the carrier and by physiologic opportunity, thus representing an example of passive delivery. This technology improves drug delivery to macrophages, increasing the amount of the drug reaching this target site, which allows reduction of the overall therapeutic dose and decrease of the adverse effects. Accordingly, the enhanced efficacy of the nanoparticle-bound antibiotics was demonstrated in a number of experimental infections (21, 22).

The potential of macrophage-targeting strategy in development of the nanoparticle-based TB drugs is supported by the *in vitro* data. Incorporation of INH and streptomycin in poly(butyl cyanoacrylate) nanoparticles not only increased the intracellular accumulation (or association) of these drugs in the cultivated human blood monocytes but also produced enhanced antimicrobial activity of these agents against intracellular *M. tuberculosis* compared with their activity in extracellular fluid (23). Similarly, the encapsulated ciprofloxacin (24) and RMP (25) produced the enhanced effect against mycobacteria in the infected macrophages. This is in contrast to the previous *in vitro* observations showing that enhanced intracellular accumulation of drugs in macrophages is rarely associated with a simultaneous increase of activity against intracellular mycobacteria (compared with activity against the extracellular bacterial population). This is presumably due to the fact that the drugs and bacteria are sequestered in different intracellular compartments. It is noteworthy that the enhanced cell uptake and activity against intracellular bacteria was demonstrated for the nanoparticulate streptomycin, an aminoglycoside agent, which, in the free form, has poor intracellular access.

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. A relatively new approach was applied to solve the problem: clofazimine was formulated as a nanosuspension consisting only of the drug and a minimum amount of surfactants (particle size, 385 nm). Intravenous injection of the nanocrystalline formulation of clofazimine resulted in a considerable reduction of bacterial loads in the liver, spleen, and lungs of mice infected with *M. avium* (26). This result correlated with the pharmacokinetic data: drug concentrations in these organs reached high concentrations, well in excess of the minimal inhibitory concentration for most *M. avium* strains. Interestingly, the effects of the nanocrystalline formulation of clofazimine were similar to those of the liposomal formulation used as a control in this study. This study is a vivid example of application of nanotechnology for overcoming the solubility problems of poorly soluble drugs. More details on the application of nanosuspensions in drug delivery can be found in a review on this subject (27).

Intravenous administration of the nanoparticles has the further advantage of passive drug delivery to inflammatory sites where the endothelium becomes permeable due to pathologic processes. In this case, passive delivery of the nanoparticles would be realized by pathophysiologic opportunity. The basic principles for engineering of the nanocarriers suggest that passive accumulation in the sites with leaky vasculature would be more effective with the long-circulating (stealth) nanoparticles (28). These particles evade resident macrophages in the liver and have higher probability to reach other sites in the body.

OTHER ROUTES OF ADMINISTRATION

Flexibility of the nanoparticle-based formulations was further demonstrated by effective subcutaneous treatment of mice infected with *M. tuberculosis* (29). A single subcutaneous dose of PLG nanoparticles loaded with RMP, INH, and PZA maintained therapeutic drug levels in plasma for 32 d and in lungs/spleen for 36 d. Moreover, this single subcutaneous injection produced a sterilizing effect in lungs and spleen of the infected mice (36 d post-treatment), thereby demonstrating a better chemotherapeutic efficacy, as compared with daily treatment using free drugs (35 oral doses). As suggested by the authors, the nanoparticles form a depot at the injection site that is slowly releasing drugs into the circulation.

CONCLUSIONS

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. The above data suggest that nanoparticles have a considerable potential for treatment of TB. Their major advantages, such as improvement of drug bioavailability and reduction of the dosing frequency, may create a sound basis for better management of the disease, making directly observed treatment more practical and affordable. Another important advantage of the nanoparticles is the feasibility of the versatile routes of drug administration, including oral and inhalation routes. In addition, high stability of the nanoparticles suggests long shelf life.

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 (30–32). These approaches would further improve efficacy and practicability of the nanoparticle-based formulations.

Finally, the success of this technology will probably depend on toxicologic issues associated with understanding of the fate of nanocarriers and their polymeric constituents in the body, as well as elimination of the risk of the residual organic solvents. In this respect, the possibility of using drug carriers made from natural polymers (e.g., chitosan or alginate) represents an attractive perspective.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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