Advanced Drug Delivery Reviews 62 (2010) 547–559

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

New old challenges in tuberculosis: Potentially effective nanotechnologies in drug delivery ☆

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ARTICLE INFO

Article history:
Received 15 May 2009
Accepted 14 September 2009
Available online 13 November 2009

Keywords:
Tuberculosis
Pharmaceutical nanotechnology
Polymeric and non-polymeric nanoparticles
Polymeric micelles and liposomes
Complexation
Local delivery to the lung
Targeting to alveolar macrophages

ABSTRACT

Tuberculosis (TB) is the second most deadly infectious disease. Despite potentially curative pharmacotherapies being available for over 50 years, the length of the treatment and the pill burden can hamper patient lifestyle. Thus, low compliance and adherence to administration schedules remain the main reasons for therapeutic failure and contribute to the development of multi-drug-resistant (MDR) strains. Pediatric patients constitute a high risk population. Most of the first-line drugs are not commercially available in pediatric form. The design of novel antibiotics attempts to overcome drug resistance, to shorten the treatment course and to reduce drug interactions with antiretroviral therapies. On the other hand, the existing anti-TB drugs are still effective. Overcoming technological drawbacks of these therapeutic agents as well as improving the effectiveness of the drug by targeting the infection reservoirs remains the central aims of Pharmaceutical Technology. In this framework, nanotechnologies appear as one of the most promising approaches for the development of more effective and compliant medicines. The present review thoroughly overviews the state-of-the-art in the development of nano-based drug delivery systems for encapsulation and release of anti-TB drugs and discusses the challenges that are faced in the development of a more effective, compliant and also affordable TB pharmacotherapy.

Contents

Abbreviations: AUC, Area-Under-the-Curve; BCS, Biopharmaceutic Classification System; CD, cyclodextrins; CFU, Colony Forming Units; CMC, Critical Micellar Concentration; DMSO, Dimethyl sulfoxide; DOTS, Directly Observed Therapy, Short course; DDS, Drug Delivery Systems; ETB, Ethambutol; FDC, Fixed Dose Combinations; HIV, Human Immunodeficiency Virus; HLB, Hydrophilic–Lipophilic Balance; HP, hydroxypropylated derivatives; HPβCD, hydroxypropyl-β-cyclodextrin; INH, Isoniazid; i.t., intratracheal; IV, intravenous; MBSA, maleylated bovine serum albumin; MDR, multidrug-resistant; MIC, Minimum Inhibitory Concentration; MMAD, mass median aerodynamic diameter; OSA, *O*-steroyl amylopectin; PAMAM, polyamidoamines; PBCA, poly(n-butylcyanoacrylate); PCL, poly(ε-caprolactone); PEG, poly(ethylene glycol); PEO–PPO, poly(ethylene oxide)–poly(propylene oxide); PIBCA, poly(isobutylcyanoacrylate); PLA, poly(lactic acid); PLGA, poly(lactic-*co*-glycolic acid); PNP, Polymeric nanoparticles; PPI, polypropylene imine; PYZ, Pyrazinamide; RAMEB, randomly methylated β-CD; RES, reticulo-endothelial system; RIF, Rifampicin; SLN, Solid lipid nanoparticles; TB, Tuberculosis; TRIPS, Trade Related Aspects of Intellectual Property Rights; WHO, World Health Organization; XDR-TB, Extremely Drug-resistant Tuberculosis.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Nanotechnology Solutions for Infectious Diseases in Developing Nations".

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doi:10.1016/j.addr.2009.11.023

1. Introduction

TB, a pervasive and deadly infectious disease of the respiratory system [1], is one of the main challenges in public health [2]. Worldwide, approximately 2 billion people are currently infected with *Mycobacterium tuberculosis*, representing about 30% of the global population [3]. After HIV/AIDS, TB is the second most deadly infectious disease [4]. In their most recent report, the WHO revealed that 9.2 million people develop the disease every year and the annual mortality rate is 1.7 million people [5]. TB is endemic in developing countries where higher mortality indexes are reported [6]. On the other hand, the infection has resurged in developed countries and due to this phenomenon the global death toll has gradually increased lasting recent years [7]. Moreover, a high prevalence of HIV/TB co-infected patients has spurred the WHO to declare a global sanitary emergency since 1993 [8].

TB infection is usually initiated by the entry of the mycobacterium to the respiratory system in aerosol droplets. Bacteria are non-specifically phagocytosed by alveolar macrophages that process the bacterial antigens and present them to lymphocytes T [9]. Then, the number of pathogens increases exponentially by killing host cells and spreading locally to regional lymph nodes in the lungs by lymphatic circulation (3 to 8 weeks after infection). Later on, dissemination of the bacilli from the infected lungs to distant highly irrigated organs (e.g. CNS, spongy bone, liver, kidneys and genitalia) takes place (3 months after infection). At this stage, acute tuberculosis meningitis or disseminated TB can sometimes result in death. The release of the bacteria to the pleura 3 to 7 months after infection results in pleurisy. Finally, extrapulmonary manifestations (e.g. lesions in bones and joints) can appear [9]. Having expressed this, a small percentage of the new cases are extrapulmonary (e.g. CNS and lymph nodes), the incidence of this TB types being dependent of the country and the subpopulation. Only 6 to 10% of HIV-negative patients develop the disease and, in most of the cases, because of the reactivation of a pre-existing infection. In contrast, HIV-infected patients have a 50 to 60% chance to show reactivation during a lifetime. Also, a fast progression toward the disease is found; higher mortalities in earlier stages of the disease have been found for HIV/TB co-infected patients [10].

Despite potentially curative pharmacotherapies being available for over 50 years, TB remains the leading cause of preventable deaths. The most effective pharmacotherapy is comprised of a multi-drug combination of INH, PYZ and RIF. During the initial intensive stage (2 months), these three agents are administered together with ETB [11,12]. The second phase (4 months) comprises exclusively RIF and INH. These four drugs together with streptomycin constitute the so-called first-line therapy (Table 1). The length of the treatment stems from the presence of (i) non-metabolizing bacteria that are not killed by the antibiotics and (ii) pathogens in stationary phase or proliferating at extremely low rates in old lesions or within fibrotic or calcified sites [13]. The prolonged pharmacotherapy and the pill burden can hamper patient lifestyle and thus low compliance and adherence to administration schedules remain the main reasons for therapeutic failure and contribute to the development of MDR strains [14]. Implementation of DOTS has been difficult, especially in developing countries and rural areas due to: (i) the difficult supervision of the regimens; and (ii) the prohibitive costs of the drugs involved, respectively [15].

Aiming to simplify administration schedules and preventing the development of mono-therapy-associated resistance to RIF, the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) recommended the application of FDC of RIF and INH plus PYZ or PYZ with ETB [16,17]; FDC combine at least 2 first-line anti-TB drugs in one single formulation (Table 1). Even though TB appears as a chronic disease with relatively slow progression, multi-resistant strains can kill immune-compromised patients in extremely short periods of time [18,19]; approximately 1–2 million patients are infected yearly with this highly aggressive type of TB. The treatment of multi-resistant TB is comprised of the administration of PYZ simultaneously with second-line drugs such as ethionamide, prothionamide, cycloserine, capreomycin, p-aminosalicylic acid or fluoroquinolones [20] (Table 1). These second-line drugs are more toxic, more expensive and less active than first-line agents. Also, prolonged treatments (9–12 months) are required in order to assure therapeutic effectiveness [9]. In this context, low patient compliance and adherence to the administration regimens become crucial drawbacks of the pharmacotherapy. Another important limitation is the variable bioavailability of several drugs. Due to the altered absorption rates under different clinical conditions (e.g., HIV), intestinal malabsorption of anti-TB drugs can be observed after oral administration in 2–5% of patients [21,22]. Among HIV/TB co-infected patients, RIF and ETB had the most pronounced reduction in intestinal absorption [9]. The limited bioavailability of RIF is a main clinical issue [23] and the WHO has expressed its concern. The drug displays a strong pH-dependent solubility (1 part in 5, 10, 250, and 360 parts of water at pH-values of 1.5, 2, 5.3, and 7.5, respectively, at 25 °C) [19,24]. Previous reports indicated that RIF displays low aqueous solubility and relatively good absorption in the stomach and classified the drug as Class II, according to the BCS [25]. However, a more recent study where the permeability of RIF was determined in three different segments of the rat intestine (employing the everted gut sac model) revealed the low intestinal absorption of RIF, suggesting a reclassification to Class IV [26]. This rectification in the BCS classification of RIF is supported by (i) the low permeability of the drug through cell monolayers; (ii) mass balance and absolute bioavailability [12]; and (iii) data according to "Lipinski's rule of five" [27]. Also, RIF is hydrolyzed to even less soluble forms such as 3-formyl rifampicin SV and 1-amino-4-methyl piperazine under acid gastric conditions. At pH-values between 7.4 and 8.2, the molecule is oxidized to an insoluble quinone derivative [14] or a desacetylated form [28]. Moreover, the absorption of RIF from FDC, usually including INH, may be substantially hindered due to the reaction between both drugs in the gastric medium to form an insoluble hydrazone [29,30].

Pediatric patients constitute a high risk population. According to WHO statistics, 250,000 children develop the disease (and approximately 100,000 die) every year [31]. Pharmacokinetics of several anti-TB drugs has shown poorer efficacy in children [32]. Also, there exist a very limited number of antituberculosis liquid formulations (Table 2) [33]. Most of the first-line drugs are not commercially available in pediatric form and they are produced only extemporaneously. For example, a liquid suspension of RIF (Rifaldin®, Sanofi-Aventis) is available in Spain. This not only results in less compliant regimens but also makes dose-per-body weight adjustments difficult. Manipulation of solid forms (e.g., crushing and mixing with food or beverages) may lead to unpredictable changes in the stability of the active compounds and their bioavailability [34].

The design of novel antibiotics attempts to overcome drug resistance, to shorten the treatment course and to reduce drug

Table 1
First and second-line antituberculosis drugs.

	Drug	Intellectual property status	Brand names (company)	Dose (mg)	Aqueous solubility (mg/mL)	BCS class
First-line drugs	Isoniazid	Generic	Rimifon® (Roche); Cotinazin® (Pfizer); Ditubin® (Schering); Nydrazid® (Bristol-Myers Squibb).	300	125	III
	Rifampicin	Generic	Rifadin® (Sanofi-Aventis); Abrifam® (Abbott); Rifaprodin® (Almirall); Rimactan® (Novartis).	300	1–2	II[a]
	Pyrazinamide	Generic	Zinamide® (Merck & Co.); Pezetamid® (Hefa-Frenon); Pyrafat® (Fatol).	500	14	III
	Ethambutol hydrochloride	Generic	Myambutol® (Dura Pharmaceuticals); Etibi, Tibutol.	400	100	III
	Streptomycin	Generic	Sesquisulfate-AgriStrep® (Merck & Co.); Streptobrettin® (Norbrook).	500	>20	N/A (i.v. or i.m.)
	Rifabutin	Pfizer	Mycobutin® (Pfizer)	150	0.19	II
Second-line drugs	Clofazimine	Generic	Lamprene® (Novartis).	100	0.01	
	Ethionamide	Generic	Trecator® (Wyeth); Nisotin®; Trescatyl® (M&B); Aetina®; Ethimide®; Iridocin® (Bayer).	500	0.1	II
	Clarithromycin	Generic	Biaxin® (Abbott); Clathromycin® (Taisho); Klaricid® (Abbott); Naxy® (Sanofi Winthrop); Veclam® (Zambon).	500	0.00033	II
	p-Aminosalicylic acid	Generic	PASER® (Jacobus); Rezipas® (Bristol-Myers Squibb).	500	1.7	Na
	Cycloserine	Generic	Closina®; Farmiserina® (Farmitalia); Micoserina®; Oxamycin® (Merck & Co.); Seromycin® (Lilly).	500	100	IV/II
	Amikacin	Generic	Sulfate-Amiglyde-V® (Fort Dodge); Amiklin®, BB-K8®, Biklin® (Bristol-Myers Squibb); Lukadin® (San Carlo); Mikavir® (Salus); Novamin® (Bristol-Myers Squibb).	1000	Na	III
	Kanamycin A	Generic	Kantrex® (Bristol-Myers Squibb).	1000	Na	N/A (i.v. or i.m.)
	Capreomycin	Generic	Capastat® (Dista), Capastat sulphate® (Eli Lilly).	1000	Soluble in water	N/A (i.v. or i.m.)
	Levofloxacin	Generic	Cravit® (Daiichi); Levaquin® (Ortho-McNeil); Tavanic® (Aventis); Quixin® (Santen).	500	Sparingly soluble in water	N/A (i.v. or i.m.)
	Moxifloxacin	Bayer	Actimax® (Sankyo); Actira® (Bayer); Avelox® (Bayer); Octegra® (Bayer); Proflox® (Esteve); Vigamox® (Alcon).	400	Na	Na
	Gatifloxacin	Kyorin Pharmaceutical Co.	Tequin® (Bristol-Myers Squibb); Zymar® (Allergan).	400	60 mg/mL at pH 4	Na
	Linezolid	Pfizer	Zyvox®, Zyvoxid® (Pfizer).	400	Soluble in water up to 3 mg/mL	Na

N/A: not applicable (administered by i.m. injection).
Na: not available.
[a] Recent reports recommend classification of RIF to Class IV [26].

interactions with antiretroviral therapies [35]; moxifloxacin (Phase III) and PA-824 (Phase II) are in advanced stages of clinical evaluation and they could be available for use by 2012 [36]. Table 3 summarizes the different drug candidates under clinical evaluation [37]. On the other hand, first generation anti-TB drugs are still effective. Overcoming the main technological drawbacks of these therapeutic agents (e.g., limited aqueous solubility and stability and bioavailability) to enhance compliance and adherence as well as improve the effectiveness of the drug by targeting the infection reservoirs (e.g., alveolar macrophages) remain the main goals of Pharmaceutical Technology. In this framework, nanotechnologies [38] appear as one of the most promising approaches [39,40]. The global nanotechnology-based drug delivery market is expected to increase from about US$3.4 (2007) to $220 billion in 2015 [41]. This is an interesting contrast as all first-line TB drugs have been marketed for 40–60 years while nanotechnology investigations of drug encapsulation and delivery are very recent.

The present review thoroughly overviews the state-of-the-art in the development of nano-based drug delivery systems for encapsulation and release of antituberculous agents and discusses the challenges that are faced in the development of a more effective, compliant and affordable TB pharmacotherapy.

2. Nanotechnologies applied to the treatment of tuberculosis

2.1. Nanodispersions

2.1.1. Nanosuspensions

Nanosuspensions are sub-micron colloidal dispersions of pure drugs stabilized with surfactants [42]. Nanonization (reduction of the average size of solid drug particles to the nano-scale generally by top milling or grinding) is a useful methodology to improve the solubility of drugs displaying strong solute–solute interactions and high melting points and, in general, both poor water and lipid solubility. The solid and dense states of the pure drug particles confer a maximal mass per volume ratio, especially critical in systems demanding high drug loadings. Despite its potential, only a few studies aiming to optimize

Table 2
Commercially available pediatric antituberculosis formulations.

Drug	Trade name (company)	Dosage forms	Dose
Rifampicin	Rifaldin® (Sanofi-Aventis)	Suspension (20 mg/mL)	Newborn (up to 1 month) 10 mg/kg/day Children 10–20 mg/kg/day Max dose 600 mg/day
Ciprofloxacin	Cipro® (Bayer)	Suspension (250, 500 mg/mL)	20–30 mg/kg/day Max dose: 1.5 g
Levofloxacin	Levaquin® (Ortho-McNeil-Janssen Pharmaceuticals, Inc.)	Solution (25 mg/mL)	10 mg/day for older children, 15–20 mg/day for younger children
Linezolid	Zyvox® (Pfizer)	Suspension (20 mg/mL)	10 mg/kg/dose every 8–12 h

Table 3
Drugs under clinical investigation for the treatment of TB.

Drug	Intellectual property status	Observations	Efficacy in humans
LL-3858 (Sudoterb®)	Lupin Ltd	Preclinical studies are in progress [Lupin website, 2006].	Web releases (for example Media coverage summary 7 2004) indicate that clinical trials may begin but the Lupin website does not post any further information on this compound.
OPC-67683	Otsuka Pharmaceutical Co., Ltd.	Patent filed in 2003. Phase I clinical trial conducted in Japan in early 2006. Results are currently unavailable. Otsuka filed a patent through the PCT process to cover 2,3-dihydro-6-nitroimidazo-(2,1-b)oxazole compounds for TB treatment.	Phase I and Phase II studies have been conducted but results are not available.
PA-824	TB Alliance and Novartis	Not approved for use in humans.	Studies are ongoing.
SQ-109	Sequella Inc.	Sequella plans an additional Phase Ib clinical study to demonstrate safety of daily administration of SQ109 alone. Then in combination with other TB drugs to evaluate safety and efficacy in patients with pulmonary TB. Additional clinical studies will begin Q2 2007.	Studies are ongoing (Phase I).
TMC-207	Johnson & Johnson	Reasonable bioavailability was found with oral solutions. Solid formulations are under development. Oral administration achieved high activity *in vivo*.	In clinical trials (Phase IIa).
FAS 20013	FASgen, Inc.	FAS 20013 is >90% orally bioavailable; it can eliminate more than99%of M. tb (including latent bacilli) within 24 h.	It will shortly enter clinical trials.

the pharmacotherapy of tuberculosis have been reported. Peters and collaborators developed a clofazimine nanocrystalline suspension in order to overcome the toxicity and the low solubility (0.3 μg/mL) of the drug [43]. To evaluate the suitability of the formulation for IV administration they compared the effectiveness of the nanosuspension in the treatment of murine *Mycobacterium avium* infection to that of drug-loaded liposomes. Application of 10-cycle discontinuous and continuous homogenization processes resulted in particles of 600 and 300 nm, respectively, both of which are small enough to prevent capillary embolism (less than 5 μm). At approximately 100 nm, the clofazimine-loaded liposomes included in the study were substantially smaller and these liposomes showed preclinical effectiveness despite limited physical stability over time, difficult reconstitution of freeze-dried samples and low drug loading capacity. An extensive size and size distribution characterization indicated that the nanosuspensions display more homogeneous size distributions, lower amounts of sub-2 and -5 μm particles and physical stability for more than 2 years. *In vivo* assays were conducted with nano-formulations containing drug concentrations between 0.16 and 0.18%. Findings showed drug concentrations above the MIC of the pathogen following the administration of the nanoparticles: 81.4, 72.5 and 35.0 mg/kg tissue in spleen, liver and lung, respectively. Moreover, continued treatment led to a significant reduction of bacterial counts in all the organs evaluated. Effectiveness levels were comparable to those of liposomal clofazimine, however, the ease of preparation and the higher physical stability of the nanosuspension were distinguishing. Reverchon et al. used supercritical carbon dioxide-assisted atomization to produce RIF sub-micronic particles of controlled size and size distribution that fit the range of injectable [44] and aerosolizable drug delivery systems (<5 μm) [45]. Of the different solvents used to solubilize the drug, DMSO was the most appropriate for nanonization. However, the high boiling point of this solvent appears as an important drawback for the scaling up. Spherical particles of sizes with mean diameters between 400 nm and 3 μm were produced. Sizes were tuned by changing the conditions of the process. Chromatographic analyses revealed that the drug did not undergo chemical degradation. These preliminary results would support further investigations to evaluate the potential of this approach as a more convenient TB pharmacotherapy, especially for the localized delivery of anti-TB drugs to the lungs (see below). A main aspect of consideration is the fact that milling might change the physical state of the drug and, by doing this, to affect its stability [46]; there are different drugs and excipients that undergo partial or total amorphization due to the exposure to mechanical forces, hence becoming less stable. Also heat production during the milling process can alter the drug form [47]. In this context, the stability of the nanonized drug needs to be thoroughly evaluated after the size reduction stage.

2.1.2. Nanoemulsions

Nanoemulsions are thermodynamically stable oil-in-water (o/w) dispersions displaying drop sizes between 10 and 100 nm [48]. A main advantage of these systems is that they are generated spontaneously and can be produced in a large scale without the need of high homogenization energy. In addition, they can be sterilized by filtration. The enhanced uptake of nanoemulsions by cells of the phagocytic system reported elsewhere renders these nanocarriers passive targeting features [49]. Also, they are up-taken by lipoprotein receptors in the liver after oral administration [50].

Ahmed and co-workers developed different o/w nanoemulsions of RIF for IV administration using pharmaceutically acceptable excipients: Sefsol® 218 as the oil phase, Tween® 80, Tween® 85 and saline water as the surfactant, the cosurfactant and the aqueous phase [51]. The mean droplet size ranged between 47 and 115 nm, the lower sizes being for systems containing lower oil contents. The entrapment efficiency was over 99% and the visual homogeneity was excellent for all the nanoemulsions. *In vitro* drug release studies indicated an initial burst effect ranging from 40 to 70% after 2 h, followed by a more moderated release afterwards. Finally, stability assays over 3 months indicated slight increases in the droplet size and the viscosity of the systems at 4 and 25 °C.

2.1.3. Niosomes

Niosomes are thermodynamically stable liposome-like vesicles produced with the hydration of cholesterol, charge-inducing components such as charged phospholipids (e.g., dicethylphosphate and stearyl amine) and non-ionic surfactants (e.g., monoalkyl or dialkyl polyoxyethylene ether) [52]. They were conceived as alternative DDS that overcome a number of drawbacks shown by the liposomes, which were mainly associated with sterilization, high production costs, scale-up difficulties and the instability of the phospholipidic

components after light exposure even at room temperature. Niosomes can host hydrophilic drugs within the core and lipophilic ones by entrapment in hydrophobic domains. In an early study, Jain and Vyas prepared micro-sized (8–15 μm) RIF-loaded niosomes containing Span 85 as the surfactant [53]. *In vivo* studies showed that by adjusting the size of the carrier, up to 65% of the drug can be localized in the lungs. In a more recent investigation, the same group of scientists extended the investigations and evaluated the biodistribution of niosomes with smaller sizes (1–2 μm) produced with different sorbitan esters (Span® 20, 40, 60, 80 and 85) and cholesterol in a 50:50 percent mol fraction ratio [54]. The entrapment extents gradually increased with the increase of the hydrophobicity of the surfactant and ranged between 20 and 35%. *In vitro* release studies showed 80% maximal and 52% minimal levels for Span-20- and Span-85-based systems, respectively; the more lipophilic the surfactant was, the slower the drug release in the aqueous medium. Niosomal formulations attained substantially higher RIF concentrations in thoracic lymph nodes via the i.p. route (46.2% of the administered dose) as opposed to 13.1% for the free drug. These findings suggested that compartmentalization of the drug took place in the lymphous tissue. In contrast, when the drug-loaded carriers were injected intravenously, only 7.3% of the drug was found in the thorax, with the accumulation extent being lower than the 11.5% obtained by free RIF. Mullaicharam and Murthy studied the organ biodistribution of RIF-niosomes (5 mg/mL) following IV and i.t. administration and compared it to that of the free drug in albino rats [55]. In general, a significant increase in the total drug concentration in the lungs, liver, kidneys and blood serum was apparent for rifampicin-loaded niosomes. After IV, niosomes preferentially 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. 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 i.t. administration, the lung/plasma ratios were 128,585 and 885 for niosomes and free drug, respectively, representing a 145-fold increase in the accumulation capacity of RIF-loaded niosomes in the lungs as compared to the free drug.

2.2. Polymeric and non-polymeric nanoparticles

PNP have been extensively explored as means for drug solubilization, stabilization and targeting [56,57]. Depending on the technology employed for their production, two kinds of systems can be generated, namely nanocapsules and nanospheres. In the former, the drug solubilized in aqueous or oily solvents is surrounded by a polymeric membrane. In contrast, the latter is comprised of solid matrices of variable porosity where the active molecules are homogeneously distributed through the particle and, often, dispersed at the molecular level. A broad spectrum of biomaterials is available for the production of PNP [58]. Advantageous features such as high stability, high loading capacity of hydrophilic and hydrophobic drugs and feasibility of administration by different routes have made PNPs one of the most popular approaches for drug encapsulation [58]. PNP are removed from the body by opsonization and phagocytosis [59]. In order to prevent recognition by the host immune system and to prolong circulation times in the blood stream, the modification of the surface with highly hydrophilic chains (e.g., polyethylene glycol) has been pursued. This approach has been one of the most extensively investigated with respect to antituberculosis drug delivery systems. Anisimova et al. investigated the encapsulation of RIF, INH and streptomycin within PBCA and PIBCA nanoparticles and tested the accumulation in human blood monocytes *in vitro* toward the development of a drug depot [60]. Encapsulated INH, streptomycin and RIF showed 4–8-, 7- and 22–25-fold increases in the intracellular concentration with respect to the extracellular concentration. In contrast, free INH and RIF showed intracellular levels similar to and 5 times higher than the extracellular concentration, respectively. Streptomycin was not detectable. More recently, moxifloxacin-loaded PBCA NPs were produced by means of the anionic polymerization of poly(butyl-2-cyanoacrylate) in the presence of the drug [61,62]. Drug encapsulation efficiency ranged between 41.0% and 47.6% and the average size was 418 nm. Un-encapsulated drug (~55%) was not removed from the formulation. *In vitro* drug release assays showed a burst effect that coincided with the unbound drug (~55%). Then, a very slow release was apparent, the total released percentage being 65% after 48 h. Cytotoxicity studies indicated that the moxifloxacin-loaded NPs were more toxic to the macrophages than the free drug [61]. When infected cells were exposed to the drug in the free and the encapsulated form, a pronounced increase in the intracellular concentration from 125–175 to 375 μg/mL was found [61]. These results relied on the NP uptake by the phagocytic cells. In addition, encapsulated moxifloxacin was more effective than the free form to kill intracellular bacilli [61]. Evaluation of the anti-TB activity in mice infected with *M. tuberculosis* showed a significant decrease in the mycobacteria count in the lungs after IV administration [62]. Regardless of the potential of this strategy, the non-biodegradability of the polymers employed constitutes a main limitation. More recently, the encapsulation of different first-line anti-TB drugs (RIF, INH and PYZ) within biodegradable PLGA nanoparticles was investigated by Khuller's group [63]. Findings showed that, as opposed to the biodistribution profile observed for the free drugs that were cleared from circulation after 12–24 h, nanoencapsulated drugs were detected in plasma for up to 9 days and therapeutic concentrations in tissues were maintained for 9–11 days. Moreover, preclinical studies in infected mice indicated that 5 oral doses of drug-loaded PLGA nanoparticles administered over 50 days effectively eliminated the pathogen from the different organs [63]. In order to attain similar results with the drugs in free form, 46 daily doses were required. Similarly, when PLGA nanoparticles simultaneously loaded with RIF, INH, PYZ and ETB were administered orally in mice and the biodistribution assayed, therapeutic levels were maintained for 5–8 and 9 days in blood and plasma, respectively [64]; one administration every 10th day (5 doses) eliminated the bacteria in the meninges (Fig. 1). Injectable PLGA NP-based implants were also administered subcutaneously in a murine model [65]. A single subcutaneous dose maintained drug plasma, lungs and spleen concentrations for more than 1 month and led to undetectable bacterial counts in the different organs. Khuller et al. also produced anti-TB drug-loaded alginate nanoparticles (235 nm diameter) by means of ionotropic gelation [66]. Encapsulation efficiency ranged between 80–90% for RIF, 70–90% for INH and PYZ and 88–95% for ETB. The nanoparticles were orally administered to mice and the plasma concentrations monitored over time. Free drugs were cleared from blood 12 to 24 h after administration and were detectable in tissues (e.g. spleen, liver and lung) only until day 1. In contrast, encapsulated drugs were observed in plasma up to 7, 9, 11 and 11 days after administration for ETB, RIF, INH and PYZ, respectively, and in tissues until day 15 [66].

Because alginate nanoparticles are produced by organic solvent-free techniques, require inexpensive equipment to manufacture and because of the stealth nature of the polymer, this method offers remarkable advantages.

SLN have longer stability and better encapsulation efficiency than liposomes and, as opposed to polymeric nanoparticles, the production process involves minimal amounts of organic solvents. RIF, INH and PYZ were incorporated into oral SLN produced by an emulsion/solvent diffusion method. Encapsulation efficiency was 51, 45 and 41%, respectively. A single dose administered orally in mice resulted in drug concentrations detectable after 3 h and for up to 8 days [67] (Fig. 2). Moreover, plasma concentrations were equal to or above the MIC at all the time points measured. The free drugs were cleared from circulation within 12 h of administration. Also, while drug-loaded SLN maintained detectable drug levels over 10 days in the lung, spleen and

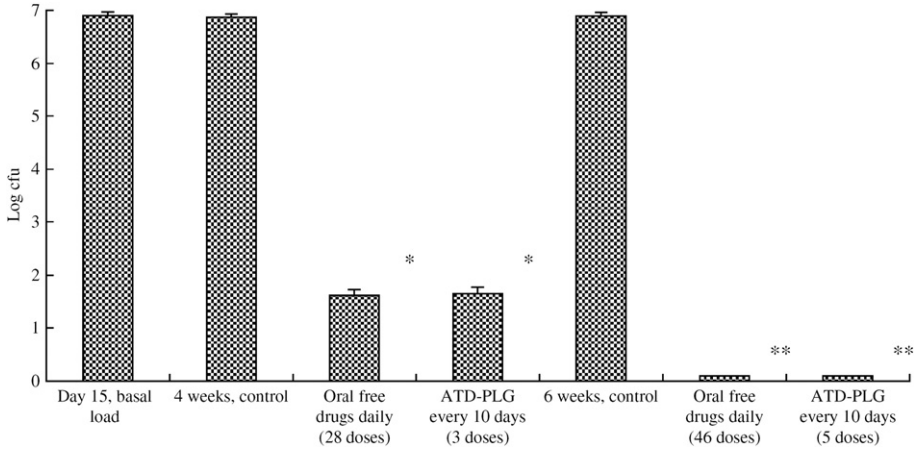

Fig. 1. Mycobacterial counts in the brain after antituberculosis treatment with drug-loaded PLGA nanoparticles in *M. tuberculosis* $H_{37}Rv$ infected mice. Values are expressed as Mean ± S.D. ($n = 5$). **No detectable CFU upon plating of undiluted tissue homogenates (Reproduced from Ref. [64] with permission from Oxford University Press).

liver, free drugs were cleared from these organs 24–48 h after the oral administration (Fig. 3). Finally, the initial CFU count 15 days after the infection with *M. tuberculosis* $H_{37}Rv$ was 4.20 and 4.34 log in lungs and spleen, respectively. Five doses of drug-loaded SLNs led to undetectable CFU. To attain a similar effect with the free drugs, 46 daily doses were required. In order to improve the intestinal mucoadhesion of the nanocarriers and the drug absorption and bioavailability, PLGA nanoparticles were surface-grafted with lectins, recognizable by glycosylated structures in the gut and the lung mucosa [68]. Plasma half life times of the different drugs encapsulated within these surface-modified nanoparticles were substantially extended from 4–9 days (uncoated) to 6–14 days upon oral/inhalation routes in mice, respectively. In addition, total bacterial clearance was achieved in lungs, liver and spleen after only 3 doses (1 every 14 days).

du Toit and collaborators developed INH-loaded polymer-based nanosystems by means of a salting-out approach (nanoprecipitation) [69]. Two kinds of precursors were used: water- or emulsion-based systems. Nanosystems appeared as spherical nanoparticles with a broad size range (77–414 nm) embedded within a micro- or nanomatrix support. The size of the nanoparticles was adjusted by changing the polymer concentration; a decrease in the concentration resulted in smaller particles. Emulsion-based nano-formulations displayed a more compact architecture. Formation of nanoparticles

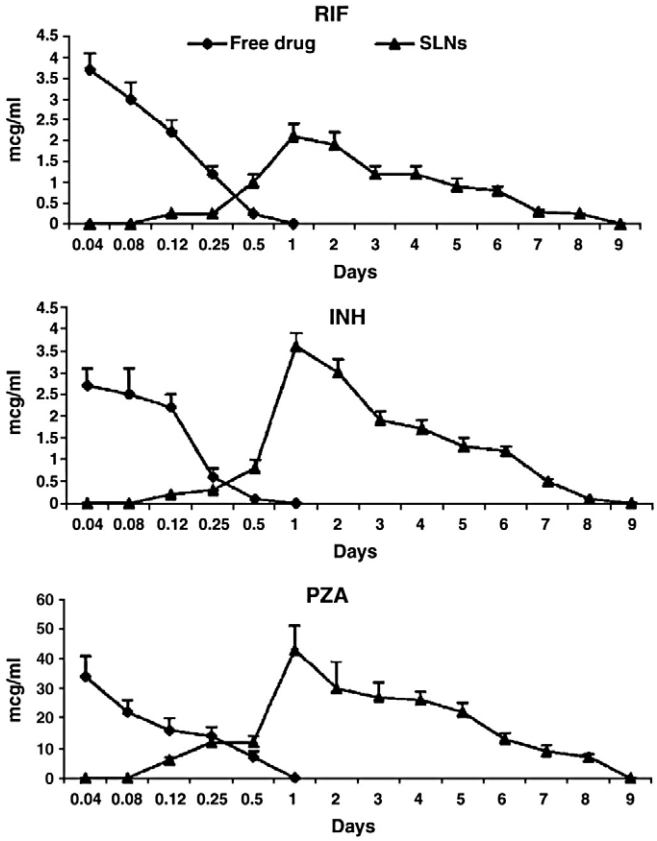

Fig. 2. Plasma drug levels after a single oral administration of drug-loaded SNL in mice. Values are expressed as Mean ± S.D. ($n = 5$). (Reproduced from Ref. [67] with permission from Elsevier).

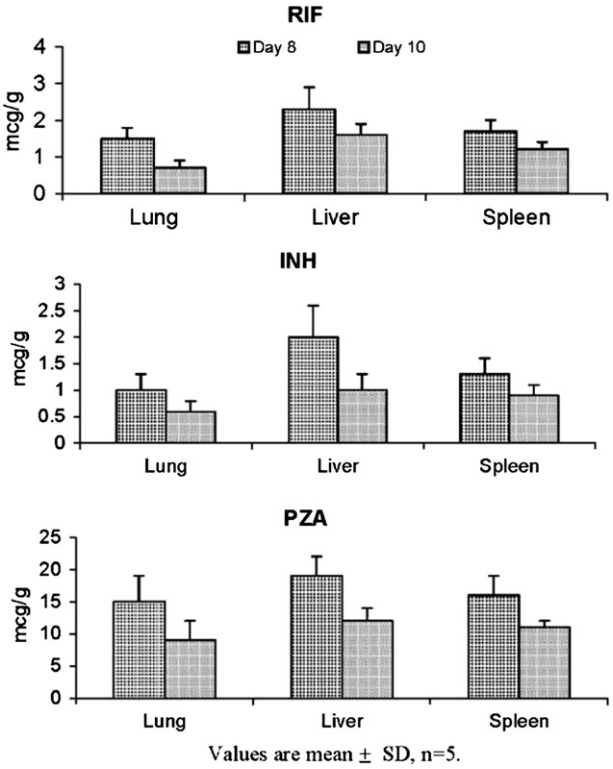

Fig. 3. Tissue drug concentrations upon a single oral administration of drug-loaded SNL in mice. Values are expressed as Mean ± S.D. ($n = 5$). (Reproduced from Ref. [67] with permission from Elsevier).

relied on the interactions of polymer chains within the polymer droplet interface involving reduction of the interfacial tension and mechanical stabilization in the dispersant electrolyte solution.

In vitro release studies indicated a bimodal profile with an initial burst effect that depended on the technique employed for the production of the nano-system, the burst being between 30–100 and 20–65% for water- and emulsion-based systems, during the first 2 h, respectively. This phenomenon was attributed to the fast initial release of INH entrapped in the outer layers of a system with a large surface area. This was followed by an exponential phase over 12 h.

2.3. Polymeric micelles and other self-assembled structures

Polymeric micelles are nanocarriers generated by the self-assembly of amphiphilic polymers in water above the CMC [70]. The hydrophilic blocks are exposed to the aqueous medium forming the micellar shell that facilitates the solubilization of the amphiphile in water and stabilizes the aggregate. In contrast, the hydrophobic blocks form the inner micellar core, a hydrophobic domain that enables the incorporation of poorly-water soluble drugs by physical interaction or chemical conjugation leading to higher solubility extents [71]. Also, sensitive drugs hosted within the core are protected from chemical and biological degradation processes. Polymeric micelles are more stable than conventional micelles (e.g., Tween® 80), even at concentrations below the CMC. This behavior stems from a relatively slow disassembly process that depends on the molecular weight and HLB of the polymer as well as on the properties of the encapsulated drug. Usually, the hydrophilic component of the amphiphile is PEG engendering the micelles with stability to withstand opzonization and elimination *in vivo* [72]. Commercially available and FDA-approved poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO) block copolymers (linear poloxamers and branched poloxamines) are among the most important micelle-forming materials [73]. Preliminary studies that investigated the solubilization of RIF within polymeric micelles of a variety of linear and branched PEO–PPO with a broad spectrum of compositions showed a minimal solubilization effect (~2-fold) [74]. These findings suggest that the size of the micellar core strongly limits the incorporation of the extremely bulky RIF molecule. Other amphiphilic block copolymers synthesized by linking mono and bifunctional PEG precursors of different molecular weight with PCL enabled the fine tuning of the HLB and the enlargement of the micellar core, improving the solubilization extent 5- to 7-fold [74]. Jiang and co-workers synthesized thermo-responsive poly(ε-caprolactone-co-glycolide)–poly(ethylene glycol)-poly(ε-caprolactone-co-glycolide) (P(CL-GA)–PEG-P(CL-GA)) smart block copolymers displaying micelle-forming and gelation properties [75]. The sol–gel transition temperature was fine tuned by changing the GA/CL ratio and the length of the hydrophobic segments. RIF-loaded (2 mg/mL) 25% gels were used to characterize the *in vitro* release profile of the matrices over time; the release was sustained over 32 days. Even though, these micellar systems did not show a substantial improvement in the solubility of the drug, they could find application in the development of a drug depot system for the sustained release of the drug.

To sustain the delivery of RIF, drug-loaded stereocomplex micelles were produced by the specific assembly of enantiomeric poly(ethylene glycol)–poly(L-lactide) (MPEG-PLLA) and poly(ethylene glycol)-poly(D-lactide) (MPEG-PDLA) block copolymers in a 1:1 ratio of L-PLA- and D-PLA-containing block copolymers [76]. CMC values of the stereocomplex micelles were lower and the sizes were smaller than those observed with the pure enantiomer-based systems. An increase in the length of the PLA segment resulted in lower CMC values and larger nano-aggregates. The RIF loading capacity and encapsulation efficiency of the stereocomplexes were higher than in enantionerically pure micelles. Drug delivery experiments *in vitro* showed a fast initial release (50% after 4–8 h) and a more moderated one (100% after 48 h) afterwards. In addition, the drug release time *in vitro* could be controlled by the polymer molecular weight. Very recently, Wu et al. designed PLA-modified chitosan oligomers capable of aggregating in water to form spherical micelles with sizes between 154 and 181 nm [77]. Incorporation of 10% RIF into the nanocarriers led to a slight core expansion to sizes in the 163–210 nm range. *In vitro* release experiments showed a burst effect (35% within 10 h) and a more sustained release until day 5. Aiming to attain higher effectiveness and longer anti-TB activity while limiting the toxic effects, Silva et al. synthesized INH-poly(ethylene glycol)–poly(aspartic acid) conjugates that sustain the release of the drug over time [78]. The micelle-forming prodrug showed a 5.6-fold increase in antituberculosous activity against *M. tuberculosis in vitro* when compared to the free drug. The mechanism would primarily involve the micelle uptake and the latter intracellular release of the drug after the hydrolysis of the linkage. The same synthetic pathway was pursued in order to encapsulate PYZ [79,80] and RIF [80]. Due to relatively low CMC values (5×10^{-4}–5×10^{-5} mg/L), micelles were stable *in vitro*. The size and distribution of the nanoparticles were found to be 78 nm, 84 nm and 99 nm, respectively, for PYZ, INH and RIF conjugates and the level of the drug conjugated was in the 65.0–85.7% range [80]. The size of the micelles would prevent renal filtration, increasing the residence times in the blood stream. Moreover, a stronger antimycobacterial activity was apparent. To overcome resistance, Jin and collaborators designed INH lipid derivatives [81]. The new amphiphilic molecules formed monolayers at the air/water interface. The aggregation behavior was intimately related to the character of the hydrophobic tail. Flexible medium-long tails formed nano-sized vesicles. In contrast, short lipid tail-derivatives displayed weak hydrophobic interactions and they did not self-assemble. Molecules with very long tails led to the formation of crystal-like structures. Finally, they showed promising antibacterial activity against *Mycobacterium* due to a more lipophilic structure that enhanced the penetration of the drug into the pathogen.

2.4. Dendrimers and other complexation strategies

Dendrimers are macromolecules displaying well defined, regularly hyperbranched and three-dimensional architecture, relatively low molecular weight polydispersity and high and adjustable functionality. Tomalia et al. developed the concept in the early 1980s with the synthesis of polyamidoamines (PAMAM), the first described dendrimers [82]. These functional molecules are feasible for drug encapsulation by virtue of the dendrimeric core and complexation and conjugation on the surface [83]. Cell-compatibility assays have demonstrated the toxicity of the amine-terminated dendrimers; the mechanism would involve the protonation of the molecule and damage to the cellular membrane [84]. Contrary to this, carboxylic- or hydroxyl-terminated dendrimers appear to be more compatible. Despite the limited usefulness of pristine PAMAMs, this conceptual approach motivated the design of surface-modified derivatives with improved performance *in vivo* [85]. Due to this unique structure, these molecules represent attractive candidates for the encapsulation and delivery of anti-TB agents for diverse administration routes, though only a few research works have been reported. To target the drug delivery to macrophages, Kumar et al. developed RIF-loaded mannosylated 5th generation (5G) PPI dendrimeric nanocarriers [86] (Fig. 4). Surface modification with sugar molecules (e.g., mannose) recognizable by lectin receptors located on the surface of phagocytic cells improved the selective uptake of the drug-loaded nanocarriers by cells of the immune system. RIF encapsulation extents were approximately 37% with hydrophobic interactions and hydrogen bonding contributing to the physical binding of the drug to the core. The solubility of RIF within unmodified dendrimers was ~52 mg/mL, while the superficial mannose molecules sterically hindered the complexation and encapsulation of the drug and the solubilization of RIF was substantially less efficient at approximately 5 mg/mL (2-fold when compared to the aqueous solubility of RIF). High haemolysis levels shown by amine-terminated

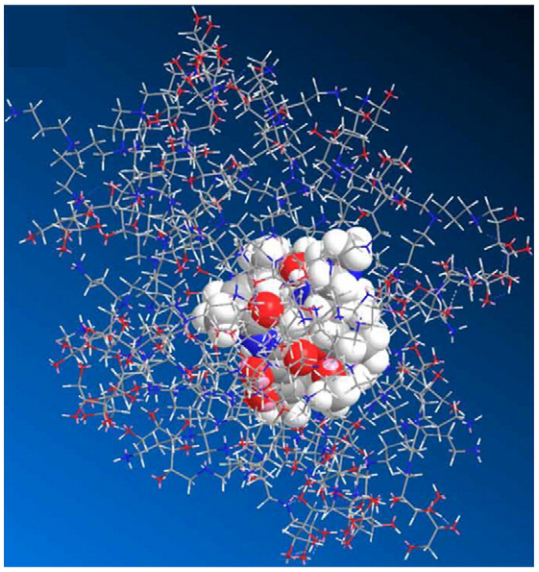

Fig. 4. Inclusion complex of mannosylated dendrimer (sticks) with RIF. Atoms are colored N, blue; C, grey; H, sky blue; O, red (Reproduced from Ref. [86] with permission from Informa Healthcare).

dendrimers precludes their clinical application. Mannosylation significantly reduced the haemolytic toxicity of the nano-carrier materials from 15.6 to 2.8%. When the RIF-containing dendrimers were assayed, findings indicated a beneficial effect of the carrier, reducing the intrinsic haemolytic effect of free RIF from 9.8 to 6.5%. A similar trend was observed when the viability of a kidney epithelial cell line was tested; encapsulation improved the survival of the cells from ~50% for free RIF to ~85%. Drug release studies conducted *in vitro* showed that the modified dendrimers sustained the release for about 120 h, as opposed to the fast delivery (<10 h) found with regular dendrimers. The phagocytic uptake of RIF and RIF-loaded dendrimers was investigated with alveolar macrophages harvested from rat lungs. A clear increase in the intracellular concentration of the antibiotic was apparent (Fig. 5).

Using a similar approach, a more recent work investigated the suitability of RIF-containing 4G and 5G PEGylated-PPI dendrimers to sustain the delivery of RIF [87]. PEGylation resulted in a significant increase of the percentage of drug entrapment from 28 and 39% to 47 and 61% for G4 and G5 derivatives, respectively. Also, the surface modification led to a better control of the release profile; i.e., 97.3 and 46.3% cumulative release values were respectively found for 4G PPI and PEG-PPI after 36 h. Finally, PEG-grafted dendrimers showed a minimal haemolytic activity (1–3%) as opposed to the NH_2-terminated ones (14–20%).

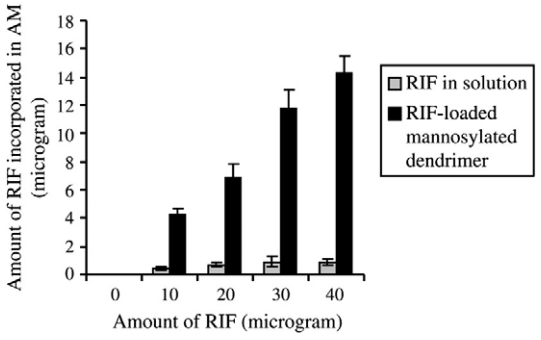

Fig. 5. Cellular uptake of RIF-loaded mannosylated dendrimers by AM cells and free RIF solutions. Values are represented as Mean ± S.D. ($n = 3$) (Reproduced from Ref. [86] with permission from Informa Healthcare).

Complexation with CD appears as another interesting approach. CD are cyclic oligosaccharides composed of 6 to 12 D-(+)-glucopyranose units linked by α-(1–4) bonds [88,89]. CD displays a toroid structure that combines a hydrophilic outer surface (due to the presence of hydroxyl moieties) and a hydrophobic inner cavity. This structure enables the partial or complete inclusion of hydrophobic molecules into the cavity; water solubility increases up to several orders of magnitude, as compared to the uncomplexed drug. DLS measurements of α-, β- and γ-CD aqueous solutions showed two size populations. One was composed of monomeric CD molecules and had a mean hydrodynamic radii <1 nm while the second was made up of self-aggregated CD molecules and was >60 nm [90,91]. Natural α-, β- and γ-CD molecules and a variety of synthetic derivatives (e.g., hydroxypropyl-γ-CD) are listed in different pharmacopeias. Several works reported on the complexation of RIF by means of different CD molecules, though results regarding the efficiency of this approach are ambiguous. Ferreira and collaborators prepared inclusion complexes of RIF with HPβCD [92]. The aqueous solubility of the drug increased linearly with the concentration of the CD, with the CD/RIF ratio in the complex being 1:1. Also, the solubilization was pH-dependent. The analysis of the chemical shift data of ^{1}H and ^{15}N NMR of free and complexed RIF revealed important changes in peaks of the side chain of the piperazine ring of the drug, suggesting the interaction of this region with the hydrophobic core of HPβCD. When RIF/β-CD complexes were prepared, UV analysis indicated the absence of strong bonding between the drug and the carrier [93]. Based on conductivity results, a stoichiometry ([CD]/[RIF]) value of 4 was determined for this complex. In addition, findings suggested that under high drug concentrations, CD induced the RIF self-aggregation. Rao et al. carried out a comparative investigation of RIF complexation with β-CD and HPβCD in order to improve the chemical stability and aqueous solubility of the drug [94]. According to phase solubility studies, 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. These results indicated a stronger interaction between the drug and the HPβCD. IR spectroscopy revealed that the interaction was through the piperazin group of RIF. However, only a 2-fold solubilization extent was apparent with β-CD when a common solvent technique was employed. As opposed to the findings by Ferreira et al. [92], herein no solubility improvement of RIF was found with HPβCD. On the other hand, all the complexes improved the thermal stability of the drug. Additionally, when the antibacterial activity was assayed in *M. tuberculosis*, a significant decrease in the MIC from 64 to 32 μg/mL was observed, this phenomenon likely due to a better permeation of the drug through the wall of the bacilli. Another drawback to the low aqueous solubility of a drug is the inability to conduct preliminary biological and clinical evaluations of new drug candidates [95]. In this context, the poorly-water soluble nitroimidazole P-824, a new anti-TB drug under study, has shown activity against drug-sensitive and multi-drug resistant bacilli. Aiming to conduct *in vivo* experiments in a short-course murine infection model, a complex with HP-γ-CD was developed and a cyclodextrin/lecithin formulation prepared [96]. A reduction in the bacterial load in the lungs was observed with 50 and 100 mg/kg doses. CD have been also investigated as carriers for local delivery to the lung [97]. They have been shown to enhance the pulmonary bioavailability of insoluble drugs while displaying high biocompatibility and minor adverse local effects (see below).

2.5. Liposomes

Liposomes are nano- to micro-sized vesicles comprising a phospholipid bilayer that surrounds an aqueous core [98]. While the core enables the encapsulation of water soluble drugs, the hydrophobic domain can be exploited to entrap insoluble agents. When administered, these carriers are recognized by phagocytic cells and are rapidly cleared from the blood stream. In order to prevent elimination

and extend circulation times, liposomes are usually PEGylated. A pioneering work explored the incorporation of gentamicin into liposomes and compared the antibacterial activity to that of the free drug in a mouse model of disseminated *M. avium* complex infection [99]. The encapsulated drug significantly reduced the bacterial count in spleen and liver. In addition, a dose-related reduction of the bacterial load, though no sterilization, was found. Similar results were obtained with different liposome-entrapped second-line antibiotics [100–102]. Deol and Khuller produced lung-specific Stealth® liposomes made of phosphatidylcholine, cholesterol, dicetylphosphate, *O*-steroyl amylopectin and monosialogangliosides/distearylphosphatidylethanolamine-poly(ethylene glycol) 2000 for the targeted delivery of anti-TB drugs to the lung [103]. Biodistribution experiments of different liposome types were conducted in healthy and tuberculosis-infected mice after IV injection. Findings showed a pronounced increase from 5.1% with conventional liposomes to 31% with PEGylated systems containing *O*-steroyl amylopectin in the accumulation of the nanocarriers in the lungs after 30 min. The accumulation extent was associated to the composition of the vesicles. Moreover, pretreatment of both healthy and infected animals with conventional liposomes (1 h before the administration of modified liposomes) saturated the reticulo-endothelial system and the uptake levels in the lungs rose to approximately 40% for the modified nanocarriers, after 30 min. On the contrary, modified liposomes showed reduced (30–50%) uptake and accumulation in the liver and spleen. Also, the biodistribution in the different organs was similar in both animal groups. The extent of drug incorporation was 8–10 and 44–49% for INH and RIF, respectively. The cytotoxicity of the drug-loaded nanocarriers was evaluated in peritoneal macrophages and compared to that of the free drugs. A significant decrease in the toxic effects was observed. This phenomenon was associated with a more controlled release of the drug. Evaluation of the hepatic activity following the administration of the free and the encapsulated drugs indicated a statistically significant decrease in the hepatoxic activity of the anti-TB agents upon encapsulation. Then, the therapeutic activity of free and encapsulated INH and RIF was evaluated in both therapeutic and sub-therapeutic dose [104]. A 12 mg/kg dose of free INH reduced the number of CFU in the lungs to about 4.5 log units, while the liposomal drug resulted in a decrease to 3.9 log units. A 10 mg/kg free and encapsulated RIF dose reduced the CFU to 4.3 and 3.8 log units, respectively. A similar trend was observed in the liver and spleen. Moreover, administration of sub-therapeutic doses (4 and 3 mg/kg for INH and RIF respectively) led to a higher decrease in CFU, as compared to the free drugs administered at a therapeutic concentration. Overall, a significant increase in the anti-TB activity was found.

With the aim of improving the anti-TB activity, reducing the toxicity and enabling the parenteral administration of highly lipophilic clofazimine, drug-loaded liposomes were produced [105] and preclinically evaluated in acute and chronic murine infections [106]. Encapsulation reduced the *in vitro* and *in vivo* toxicity of the drug and enhanced the anti-TB activity in both acute and chronic models. This inhibition was markedly higher in the liver and lung. In addition, chronically infected mice treated with the encapsulated drug showed total clearance from the liver and spleen with no signs of recovery 2 months post-treatment. In the lungs, a gradual decrease in CFU was observed, though a rebound was found 2 months post-treatment. Regardless of the apparently total clearance of the bacilli following a second treatment with the liposomal drug, a similar phenomenon was observed after 2 months. In more recent investigations, PYZ- [107] and rifabutin-containing liposomes [108] were also produced, stressing the great versatility and potential of these nanocarriers.

3. Local delivery of anti-TB drugs to the lung and targeting strategies

Since most of the TB manifestations are observed mainly in the respiratory system, local delivery to the lungs by inhalation has emerged as one of the most attractive administration routes to target the TB infection's cellular reservoir (alveolar macrophages), while reducing systemic adverse effects [109]. Nanotechnology platforms (e.g., polymeric nanoparticles and liposomes) that were previously investigated with the aim of optimizing different technological aspects of anti-TB drugs are currently being explored for the targeted delivery to the lungs [110,111]. In this framework, different industrial technologies such as spray drying are being employed to produce nanoparticles made of a variety of natural (e.g., gelatin) and synthetic (e.g., polybutylcyanoacrylate) polymers [112]. The first part of the present section overviews the cornucopia of nanocarriers explored for the local administration of anti-TB drugs. Afterwards, targeting strategies by systemic route are overviewed. In one approach, RIF-, INH- and PYZ-loaded PLGA nebulizable nanoparticles (186–290 nm diameter) were produced by a multiple emulsion methodology [113]; the drug encapsulation extents were 56.9%, 66.3% and 68% for RIF, INH and PYZ, respectively. A MMAD of 1.88 μm indicated that the nanoparticles could be respired. Nebulization of drug-loaded nanoparticles resulted in plasma-detectable concentrations after 6 h. In addition, therapeutic drug concentrations were detected until day 6 for RIF and day 8 for both INH and PYZ. Pharmacokinetic experiments indicated that the C_{max} of encapsulated RIF and PYZ administered by nebulization were similar to those attained by the oral route with free drugs though the time to the peak concentration was longer for the nanoparticles. On the other hand, higher AUC was found due to a more prolonged $t_{1/2}$. Bioavailability values increased 12.7- and 6.5-fold for RIF, 19.1- and 32.8-fold for INH and 14.7- and 13.4-fold for PYZ as compared to the IV and oral bioavailability of the free drugs, respectively. Bioavailability levels increased more pronouncedly when the comparison was done with the free drugs administered by nebulization: 51.0-, 20.0- and 28.0-fold for RIF, INH and PYZ, respectively. Moreover, as opposed to the free drugs that were undetectable after 24 h, encapsulated drugs were detected in the lungs until day 11. Finally, five doses of nanoparticles administered every 10 days showed the same anti-TB effectiveness as 46 oral daily doses. Anti-TB-loaded alginate nanoparticles initially developed for systemic administration [66] were also evaluated for local delivery to the lungs [114]. When the drug-loaded alginate nanoparticles (MMDA 1.1 μm) were administered by nebulization, drug concentrations were detected in the plasma after 3 h, which was faster than PLGA nanoparticles [114]. Also, INH, RIF and PYZ were detectable up to 14, 10 and 14 days, respectively, as opposed to the fast clearance of the free drugs after 12–24 h. Nebulization of drug-loaded alginate nanoparticles (1 dose biweekly, 3 doses) over the course of 45 days was as effective in clearing the lungs and the spleen of *M. tuberculosis*-infected guinea pigs as 45 oral daily doses of the free drugs. Ohashi et al. produced RIF-loaded biodegradable PLGA that were incorporated into mannitol microspheres in one single step by means of a four-fluid nozzle spray drier [115]. Encapsulation of the nanoparticles in mannitol improved the *in vivo* uptake of the drug by alveolar macrophages in rat lungs as compared to RIF-containing PLGA and mannitol microparticles. Thus, it appears as a potentially efficient DDS for the treatment of TB.

Vyas et al. developed RIF-containing aerosolized micrometric liposomes to target the alveolar macrophages, which is a prevalent infection site [116]. They anchored MBSA and OSA to the surface of the nanocarriers with the intention to improve the selectivity for the lung; the former is recognized by macrophage scavenger receptors and the latter shows affinity for alveolar macrophages. Liposomes were formulated in chlorofluorocarbon propellants and packed in a pressurized container. *In vitro*, 1.5–1.8 fold higher penetration extents were discovered, as compared to an RIF aerosol. Anti-TB assays with *M smegmatis*-infected macrophages showed a significant decrease in bacterial viability from 45.7 to 7–11% for the ligand-modified liposome and the free drug, respectively. Unmodified liposomes showed intermediate activity with 31.6% viability, while controls showed 69.5% viability. *O*-steroyl amylopectin-coated liposomes were more

effective than MBSA, likely due to better accumulation in alveolar macrophages. Biodistribution showed that all the liposomes, independently of the modification, led to higher concentrations in the lungs and lower concentrations in the plasma when compared to the free drug. For example, after 30 min, lung and serum accumulation of RIF encapsulated in MSBA and OSA liposomes was 61.5% and 11.3%, and 65.1% and 9.2% of the initial dose, respectively. Contrary to this, the free drug showed percentages of 39.1% and 29.8% for the lungs and plasma, respectively. Remarkably, 10.8% of OSA liposomes were retained in the lungs after 24 h as opposed to 8.2% with MBSA-modified ones. These systems are in the micrometric scale, however, limitations such as leakage of the drug during the administration, fast clearance from the blood and the undesired uptake by RES after the IV administration of liposomes and other nanocarriers are overcome. Thus, they appear as an interesting platform toward the selective delivery of drugs to the respiratory system.

Metilainen et al. extensively evaluated the cytotoxicity *in vitro* and the permeation across Calu-3 cell monolayers of a broad spectrum of natural and synthetic CD molecules, including α-, β-, γ-, HP and RAMEB [117]. CD molecules usually interact with the cellular membrane, removing essential components. CD treatments did not affect the viability of cells *in vitro* when concentrations were <1 mM. At higher concentrations, a more diverse response was found. When these responses are considered, the HP-CD treatment is the safest derivative. Cell layers were more resistant to the toxic effect of the CD molecules due to the formation of tight junctions. Evaluation of drug permeability through a pulmonary epithelium cell monolayer model suggested that CD molecules cross the cell layer by passive diffusion through a paracellular route. Accordingly, water solutions of HPβCD, RAMEB and γ-CD were used to produce breathable aerosol droplets with size ranges that were compatible with pulmonary deposition. Moreover, the short-term exposure to inhaled CD solutions was non-toxic. Based on these findings, Tewes and co-workers investigated the solubilization of RIF in 5% HPβCD and RAMEB water solutions under different pH conditions (4, 7.4 and 9) [118]. At pH 4, CD ineffectively solubilized the drug. Solubility gradually increased with RAMEB as the pH rose, being two times higher at pH 7.4. At pH 9, a more pronounced solubilizing effect was apparent for both derivatives. According to phase solubility studies conducted at pH 9, a 1:1 complex was hypothesized for RAMEB in the 0–0.23 M range. The HP counterpart showed a linear increase in the drug solubility which was also consistent with a 1:1 ratio, though only between 0 and 0.066 M. Then, a plateau for higher CD concentrations indicated the saturation of the solution in both free and complexed drug. In order to enable nebulization, the pH of the solutions needs to be physiological. A 1:6 dilution of the RIF/RAMEB complex in pH 7.4 buffer displayed physical stability for at least 2 days. Aiming to evaluate the ability of RIF/CD complexes to permeate across the alveolar epithelium, the diffusion through a Calu-3 cell monolayer (an epithelial alveolar model with a high level of tight junctions) was measured. Findings showed that complexation slightly reduced (1.4-fold) the transcellular diffusion-driven transport. A linear increase in the flux versus the total RIF concentration was attributed to the consequent increase in the concentration of free drug. The RIF flux from RAMEB complexes containing 21.6 mM drug concentration was comparable to that of a saturated solution of free RIF (2.6 mM). These results suggested that a complex containing >21.6 mM RIF should serve as an efficient delivery system. The bacteriostatic activity of the RIF/CD complexes was evaluated by assaying the MIC in *A. baumannii*. While RIF/RAMEB (MIC = 17.5–35 μg/mL) showed an equivalent activity to the free drug (MIC = 10–20 μg/mL), RIF/HPβCD showed a much higher effect as the MIC was discovered to be between 3.75 and 7.5 μg/mL. Finally, nebulization efficiency of both drug-loaded systems was investigated. RAMEB showed a lower efficiency than a PBS solution. In contrast, for RIF/HPβCD, it was significantly higher and the respirable fractions were approximately 70% for all formulations. Furthermore, the MMAD of the hydroxypropyl complexes was significantly smaller than those observed for free RIF and RIF/RAMEB, enabling the eventual delivery of the drug deep into the lungs.

Systemically-administered nanocarriers to target the lungs have been also intended, though this strategy appears as a less preferable due to its invasiveness and the potentially harmful systemic exposure to the antibacterial agents. In previous sections, different nanocarriers displaying preferential accumulation in lungs and other organs have been described [53–55]. However, the mechanism involved in the uptake is passive targeting due to the fact that surface-unmodified nanoparticles stay relatively unspecific vectors. In this context, nanocarriers bearing surface modifications to specifically target alveolar macrophages following pulmonary and other administration routes have been investigated; i.e., sugars such as mannose that are recognized by lectin receptors sited on the surface of macrophages [119,120]. Chono et al. developed mannose-coated liposomes (1000 nm) and compared the uptake by rat alveolar macrophages (AM) *in vitro* with that of unmodified liposomes (100 to 2000 nm) [121]. A pronounced increase in the uptake was observed with the mannosylated-nanocarriers. In addition, greater accumulation of modified liposomes was found in the lungs after pulmonary administration to rats. More recently, ciprofloxacin-loaded mannose-modified liposomes were prepared and the performance *in vivo* after inhalation was evaluated [122]. Encapsulation of the drug into mannosylated liposomes led to a sharp increase in the uptake by AM from approximately 12% to 22% of dose per mg of cell protein. In addition, biodistribution assays indicated 1.5- and 2.4-fold greater AUC and maximum concentration values, respectively, with the modified liposomes. Also, plasma levels were reduced by encapsulation as compared to the free drug. Finally, a preferential uptake of the liposomes by pulmonary macrophages was apparent, this behavior being more noticeable for the mannose-coated liposomes. In a similar approach, Wijagkanalan et al. studied the *in vitro* and *in vivo* uptake of liposomes (90–125 nm) bearing increasing concentrations of mannose on the surface [123]. The higher the mannose concentration on the surface, the more pronounced the cellular uptake. Also, the uptake of modified liposomes *in vitro* was inhibited with an excess of solubilized mannan, confirming the specificity of the carrier–receptor interaction. Accumulation in alveolar cells after intratracheal administration to rats indicated the higher uptake of mannosylated-nanocarriers, preferably (15–17-fold) by alveolar macrophages over alveolar epithelial type II cells. Other works evaluated the targeting by means of pulmonary surfactant-grafted liposomes [124]. However, the uptake by alveolar epithelial type II cells was approximately 2 to 3 times higher than that of alveolar macrophages after intratracheal administration in rats [125].

4. Ethical issues and perspectives

New drug design and development by the pharmaceutical industry usually focuses on diseases affecting affluent populations (e.g., cancer) that can afford expensive new drugs and medicines coupled to more sophisticated technologies [126]. Infectious and parasitic diseases are expected to remain a lethal problem in developing and underdeveloped countries [127]. In this context, one-third of destitute people (particularly those in Africa and India) do not have access to essential medicines [128]. Tuberculosis infection has a greater impact on developing countries yet the development of first-line drugs has virtually stalled; they have remained unchanged for four decades [129]. TB is notorious for being the first cause of preventable death, yet efforts to overcome the obvious fails current drug treatments, namely low aqueous solubility and stability, limited bioavailability and low patient compliance, have become complacent. On the other hand, the appearance of HIV-associated and XDR-TB in developing countries led to a resurgence of new drug and DDS research to shorten the 6-month treatment period and to improve patient compliance and adherence to the regimens [128]. Amid these dilemmas, agreement exists that profit-motivated R&D precludes the poor [126].

In light of these issues, the emerging ethical issues can be separated into two levels. The first level is related to the direct access to conventional anti-TB formulations. The multilateral agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) was built to protect the interests of pharmaceutical companies and has set minimal standards for Intellectual Property law-making in the countries that adhered to the agreement. Since its implementation in 1995, patent protection has limited access to life-saving medicines. This is due to high drug prices and the unwillingness to invest R&D in "unprofitable" diseases. Recognizing these problems, the 2001 Doha Declaration on TRIPS stated that IP protection laws should not be applied in cases where the agreement could lead to a serious damage to public health [130]. A compulsory license (CL) is a license given by a State upon the request of a third party (another pharmaceutical company that produces generic medicines) that allows the generic producer to use a patented invention (e.g., active pharmaceutical ingredient) without the express consent of the patent holder. For example, this can be used in cases when the patent holder does not supply the medicine to a market due to the non-profitability of the medicine, which makes it a useful tool for reducing the price of certain drugs. As opposed to the increasingly expensive anti-HIV therapy, first-line anti-TB drugs have long been on the market and can further become accessible if such a license is granted to a country in need [126]. A different perspective is apparent in the case of the more expensive second-line medicines used in MDR-TB infections. Here, a six-step process has been implemented to make the drugs affordable [20]; the supply of quality-assured second-line drugs increased and prices decreased. Moreover, some countries are purchasing the active pharmaceutical ingredients from quality-assured generic manufacturers. For example, the price of ofloxacin is up to 8-fold higher in countries where the drug is patented than in places where it is not [20]. Unfortunately, it can be argued that such a decline in price could harm the development of innovative medicines as they become less profitable [130].

The second level pertains to the restricted access to cutting-edge technology formulations (e.g., DDS, targeted medications) due to substantially more expensive prices. It is still unclear whether the design and development of more advanced anti-TB medicines will benefit broad portions of the infected population or only the more affluent groups of patients in developed countries. Overall, the challenges ahead demand the design of DDS formulations that address the different limitations of the anti-TB pharmacotherapy and, in addition, make them affordable to all patients, regardless of their socioeconomic status.

Acknowledgements

We thank Omar F. Khan for the critical revision of the manuscript. RJG and MAM thank PhD scholarships of the CONICET.

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