Nanotechnology Based Approaches for Tuberculosis Treatment

Edited by Prashant Kesharwani

NANOTECHNOLOGY BASED APPROACHES FOR TUBERCULOSIS TREATMENT

Page left intentionally blank

NANOTECHNOLOGY BASED APPROACHES FOR TUBERCULOSIS TREATMENT

Edited by

PRASHANT KESHARWANI

Assistant Professor & Ramanujan Fellow, Department of Pharmaceutics,
School of Pharmaceutical Education and Research
Jamia Hamdard (Hamdard University)
New Delhi, India

ACADEMIC PRESS

An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-819811-7

Publisher: Andre Gerhard Wolff
Acquisitions Editor: Erin Hill-Parks
Editorial Project Manager: Pat Gonzalez
Production Project Manager: Maria Bernard
Designer: Miles Hitchen

Typeset by Thomson Digital

Contents

Contributors

Suneera Adlakha Institute of Nano Science and Technology (INST), Mohali, Punjab, India

Zahoor Ahmad Infectious Diseases PK/PD Lab, Life Science Block, Indian Institute of Integrative Medicine, Srinagar, Jammu and Kashmir, India

Ravi Bandaru Department of Industrial and Engineering Chemistry, Institute of Chemical Technology, Indianoil Odisha Campus, Bhubaneswar, India

Simone Pinto Carneiro Laboratory of Phytotechnology, School of Pharmacy, Federal University of Ouro Preto (UFOP), Ouro Preto, Minas Gerais, Brazil

Laura E. Carreto-Binaghi Department of Research in Microbiology, National Institute of Respiratory Diseases, Mexico City, Mexico

Hira Choudhury Department of Pharmaceutical Technology, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia

Rambabu Dandela Department of Industrial and Engineering Chemistry, Institute of Chemical Technology, Indianoil Odisha Campus, Bhubaneswar, India

Neelam Dhankar Department of Pharmaceutical Sciences, Starex University, Gurugram, Haryana, India

Orlando David Henrique dos Santos Laboratory of Phytotechnology, School of Pharmacy, Federal University of Ouro Preto (UFOP), Ouro Preto, Minas Gerais, Brazil

Damián Eduardo Pérez-Martínez Health Sciences Doctoral Program, Health Sciences Institute, University of Veracruz, Xalapa, Veracruz, México

Zohreh Firouzi Department of Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Ashish Kumar Garg Akal College of Pharmacy and Technical Education, Mastuana Sahib Barnala Road, Mastuana Sahib, Punjab, India

Yolanda Gonzalez Department of Research in Microbiology, National Institute of Respiratory Diseases, Mexico City, Mexico

Bapi Gorain School of Pharmacy, Faculty of Health and Medical Sciences, Taylor's University, Subang Jaya, Selangor, Malaysia

Silvia Guzmán-Beltrán Department of Research in Microbiology, National Institute of Respiratory Diseases, Mexico City, Mexico

Mayank Handa Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research—Raebareli, Lucknow, India

Ali Ibrahim Bekraki Faculty of Pharmacy, Jinan University, Tripoli, Lebanon

Esmeralda Juárez Department of Research in Microbiology, National Institute of Respiratory Diseases, Mexico City, Mexico

Prashant Kesharwani Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard (Hamdard University), New Delhi, India

Farzad Khademi Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

Mradul Mohan ICMR-National Institute of Malaria Research, New Delhi, India

Nagashekhara Molugulu School of Pharmacy, Monash University, Jalan Lagoon Selatan, Bandar Sunway, Selangor, Malaysia

Mohamad Mosa Mubarak Infectious Diseases PK/PD Lab, Life Science Block, Indian Institute of Integrative Medicine, Srinagar, Jammu and Kashmir, India

Ramakanta Naik Department of Engineering and Materials Physics, Institute of Chemical Technology, Indianoil Odisha Campus, Bhubaneswar, India

Bernadette Dian Novita Department of Pharmacology and Therapy, Faculty of Medicine, Widya Mandala Catholic University Surabaya, Surabaya, Indonesia

Rupal Ojha Department of Biochemistry, Central University of Rajasthan, Ajmer, Rajasthan, India

Rajan Kumar Pandey Department of Biochemistry, Central University of Rajasthan, Ajmer, Rajasthan, India

Sreenivas Patro Sisinthy School of Pharmacy, Faculty of Health and Medical Sciences, Taylor's University, Subang Jaya, Selangor, Malaysia

Vijay Kumar Prajapati Department of Biochemistry, Central University of Rajasthan, Ajmer, Rajasthan, India

Amal Rabti Sensors and Biosensors Group, Laboratory of Analytical Chemistry and Electrochemistry (LR99ES15), Department of Chemistry, University of Tunis El Manar, Tunis, Tunisia

Amal Raouafi Sensors and Biosensors Group, Laboratory of Analytical Chemistry and Electrochemistry (LR99ES15), Department of Chemistry, University of Tunis El Manar, Tunis, Tunisia

Noureddine Raouafi Sensors and Biosensors Group, Laboratory of Analytical Chemistry and Electrochemistry (LR99ES15), Department of Chemistry, University of Tunis El Manar, Tunis, Tunisia

Eupa Ray Institute of Nano Science and Technology (INST), Mohali, Punjab, India

Deviprasad Sahoo Department of Engineering and Materials Physics, Institute of Chemical Technology, Indianoil Odisha Campus, Bhubaneswar, India

Aakriti Sethi Department of Chemistry and Applied Biosciences, ETH Zurich, Zürich, Switzerland

Noorsuzana Mohd Shariff Advanced Medical and Dental Institute, Universiti Sains Malaysia, Kepala Batas, Penang, Malaysia

Ankur Sharma Institute of Nano Science and Technology (INST), Mohali, Punjab, India

Farideh shiehzadeh Department of Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Rahul Shukla Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research—Raebareli, Lucknow, India

Sima Singh Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Mohsen Tafaghodi Department of Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences; Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Pushpendra K. Tripathi Rameshwaram Institute of Technology and Management, Lucknow, India

Kalpesh Vaghasiya Institute of Nano Science and Technology (INST), Mohali, Punjab, India

Rahul Kumar Verma Institute of Nano Science and Technology (INST), Mohali, Punjab, India

Roberto Zenteno-Cuevas Public Health Institute, University of Veracruz, Xalapa, Veracruz, México

Preface

Tuberculosis (TB) is a leading chronic bacterial infection. Despite potentially curative pharmacotherapies being available for over 50 years, the length of the treatment and the pill burden can hamper patient lifestyle. Prolonged treatment, high pill burden, low compliance, and stiff administration schedules are factors that are responsible for the emergence of multidrug-resistant strains. According to WHO reports, 53 million TB patients died from 2000 to 2016. Therefore, early diagnosis of the disease is of great importance for global health care programs. Various unique antibodies have been developed to overcome drug resistance, reduce the treatment regimen, and elevate the compliance to treatment. Therefore, we need an effective and robust system to subdue technological drawbacks and improve the effectiveness of therapeutic drugs which remains a major challenge for pharmaceutical technology.

Regarding TB treatment, nanoparticles can be a useful strategy for two distinct applications: (1) for their intrinsic antimycobacterial activity and (2) as vehicles for known antitubercular drugs to allow the reduction of dose- and drug-associated side-effects and administration via user-friendly administration routes such as pulmonary or oral ones.

This book will summarize the types of nanodrugs, their synthesis, formulation, characterization, and applications, with the most important administration routes. Thus, this book will discuss various nanotechnology-based approaches which may help overcome persisting limitations of conventional/traditional treatment. Also, recent advances and achievements regarding therapeutic efficacy provide possible future applications in this field. In this scenario, this book will directly address all translational aspects and clinical perspectives of TB nanomedicine from a comprehensive and multidisciplinary perception. This book is thus (1) an unrivalled, comprehensive summary of the field and (2) rationally conceived clinical stage of TB nanomedicines. The editor and contributors (authors) cover a wide range of expertise in the nanomedicine and TB and all of them are already proven their international acclaim.

We thank all the authors for their valuable and timely contributions. We believe that the book, with its comprehensive coverage of fundamental and applied aspects of the subject, will prove immensely useful to its readers and stimulate further interest.

Prashant Kesharwani

CHAPTER

15

Nanotechnology approach in conquering anti-TB resistance

Bernadette Dian Novita

Department of Pharmacology and Therapy, Faculty of Medicine, Widya Mandala Catholic University Surabaya, Surabaya, Indonesia

Abbreviations

AFB Acid Fast Basil
MDR-TB multidrug resistant tuberculosis
M.tb *Mycobacterium tuberculosis*
TB tuberculosis
XDR-TB extra-drug resistant tuberculosis
RIF rifampicin
INH isoniazid
PYR pyrazinamide
ETB ethambutol

1 Mycobacterium: pathogenesis and its problem in the resistant

Tuberculosis (TB) is an air-borne chronic infection caused by *Mycobacterium tuberculosis* (M.tb). It has a high affinity to the parenchymal tissue of lungs due to the high oxygen level. *Mycobacterium tuberculosis* (M.tb) is a Gram-positive acid-resistant stem and called Acid Fast Basils (AFB). The golden standard in identified TB infection is the count of AFB smears from sputum, for TB in the lung or other specimens, for extrapulmonary TB [1].

Pathogen M.tb is easily to die with direct sunlight. However, it survives in dark and damp places, even it is outside the host. *Mycobacterium tuberculosis* transmits through aerosol droplets from coughing, sneezing, or saliva splashes of people infected TB lungs. The droplet diameter is very small (0.5-5 μm) and around 40,000 bacteria are produced each sneezing, therefore M.tb eases to transmitted [1].

After the inhalation of *M. tuberculosis*, innate immune responses involving alveolar macrophages and granulocytes begin to combat the infection; in some persons, the bacilli are cleared, whereas in others, infection is established [2]. The replication of bacilli in macrophages and regional lymph nodes leads to both lymphatic and hematogenous dissemination, with seeding of multiple organs, which may eventually give rise to extrapulmonary disease. The containment of bacilli within macrophages and extracellularly within granulomas limits further replication and controls tissue destruction, resulting in a dynamic balance between pathogen and host. The classic interpretation of this as a binary

http://dx.doi.org/10.1016/B978-0-12-819811-7.00015-1

process with either truly latent *M. tuberculosis* infection or active tuberculosis disease has recently been challenged as an oversimplification. Instead, a spectrum of immunologic responses that are both protective and pathogenic and correlate with a range of bacterial activation has been suggested. This continuum encompasses a variety of host-microbe interactions, which are characterized by clinical latency when host responses predominate and by disease when bacterial replication exceeds the threshold required to cause symptoms [2-4]. Recent evidence suggests that host inflammatory responses, particularly with interleukin-1β, may actually enhance mycobacterial replication, which illustrates that the double-edged sword of immune responses seen in tuberculosis disease may also be present in latent infection (as shown in Fig. 15.1).

Mycobacterium tuberculosis forms into an active, clinically silent, and latent infection. It said that one-third of the world's population infected by M.tb, most of them are asymptomatic and become latent tuberculosis infection especially in people with immunocompromised conditions, for example, HIV/AIDS, DM, malnutrition, on chemotherapy or steroids therapy, and antitumor therapy necrosis factor. Only about 5%-10% suffer from active tuberculosis infection [1].

Tuberculosis (TB), the infection caused by *Mycobacterium tuberculosis* (M.tb), remains a problem to overcome in Indonesia. In East Java Provence Indonesia 2018, the incidence of new TB cases reached 767 from 100,000 population [6]. This phenomenon was similar to TB incidence in the world. According to the World Health Organization (WHO) data in 2013, it states that the incidence of new TB cases in the world has increased 50%, and therefore WHO has declared for TB as a "global health emergency" [7]. The pathology mechanism of TB could be seen in Fig. 15.2.

Mycobacteria, especially *Mycobacterium tuberculosis* (M.tb), are intrinsically resistant to most antibiotics [9]. They have the ability in growing slower than other bacteria. There is no single antibiotic that is relatively effective against M.tb, therefore for tuberculosis (TB) multidrugs therapy (MDT) is required to avoid bacterial resistant [9,10]. Mycobacterial cells are lipid-rich and also able to be dormant that causes impermeable and poorly penetrate for many agents, including macrophages.

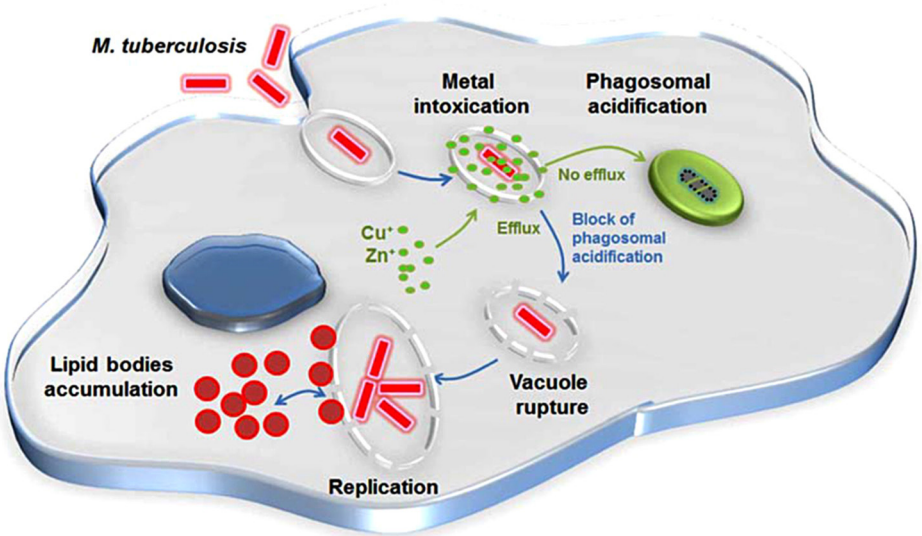

FIGURE 15.1 **The main subcellular events of *M. tuberculosis* infection in macrophage [5].**

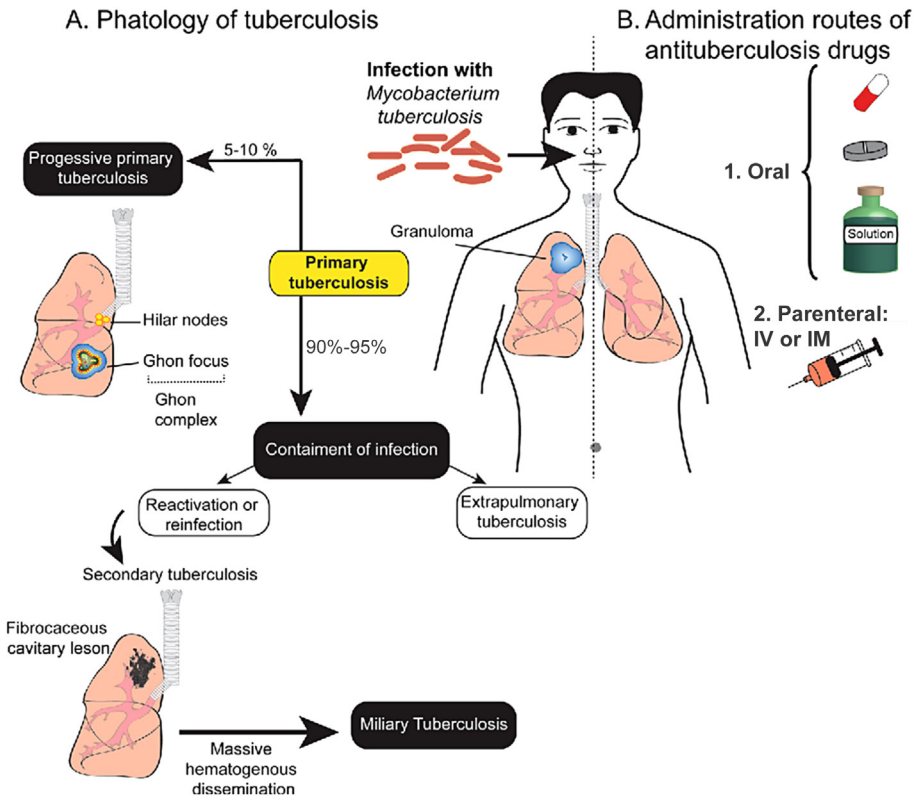

FIGURE 15.2 Pathology of tuberculosis [8].

Finally, *M. tuberculosis* has the ability to manipulate both innate and adaptive immune response and called TB's escape mechanism. In this mechanism, M.tb has a high ability to avoid intracellular killing process and macrophage phagocytosis process [11,12]. Mycobacteria are notorious for their ability to develop resistance [9,10]. Moreover, phagosome maturation, which is activated during M.tb recognition process, is the decrease of intra-vacuole pH, from ~7 to 5. This acidification represents a fundamental blocking step in the process of bacterial elimination [5].

The worldwide emergence and spread of drug-resistant *M. tuberculosis* strains is a serious concern considering the high death rate associated with such infections. To tackle this issue, new therapeutic molecules and vaccination/prevention strategies have to be developed. However, such developments may require a better understanding of how *M. tuberculosis* blocks the innate defenses of the host to establish its intra-cellular replicative niche [5].

2 Antituberculosis and the mechanism of antituberculosis resistant

The aims of antituberculosis (anti-TB) are (1) to cure the patient; (2) to prevent death; (3) to prevent recurrence; (4) to break the chain of transmission; and (5) to prevent M.tb resistant. Mycobacterium has ability to grow very slowly and develop resistance rapidly. Therefore, to treat TB, several combinations or TB-MDT are needed.

Tuberculosis MDT is classified into two lines: the first line of TB MDT is rifampicin (RIF),

isoniazid (INH), pyrazinamide (PYR), ethambutol (ETB), and streptomycin. This group of drugs exhibits high effectiveness with acceptable toxicity [9,10,13]. The second line of TB-MDT is the antibiotics fluro-quinolones (such as ciprofloxasin, ofloxasin, levofloxasin, mofifloxacin), macrolides (such as erythromycin, clarithromycin), and aminoglycosides (such as amikacin, kanamycin, and capreomycin) [9,10,13].

The aims of TB-MDT are (1) to increase bactericidal activity, starting from the beginning of therapy phase; (2) to prevent drug resistant; and (3) to enhance the process of eliminating *M. tuberculosis* in the sites of TB infection [9,10,13].

Isoniazid is the primary drug for chemotherapy of TB infection with the highest bactericidal activity at the beginning of TB treatment. All patients infected with isoniazid-sensitive strains of the tubercle bacillus should receive the drug if they can tolerate it. The use of combination therapy (isoniazid + pyrazinamide + rifampin) provides the basis for "short-course" therapy and improved remission rates [9,10]. The mechanism of action of anti-TB could be seen in Fig. 15.3.

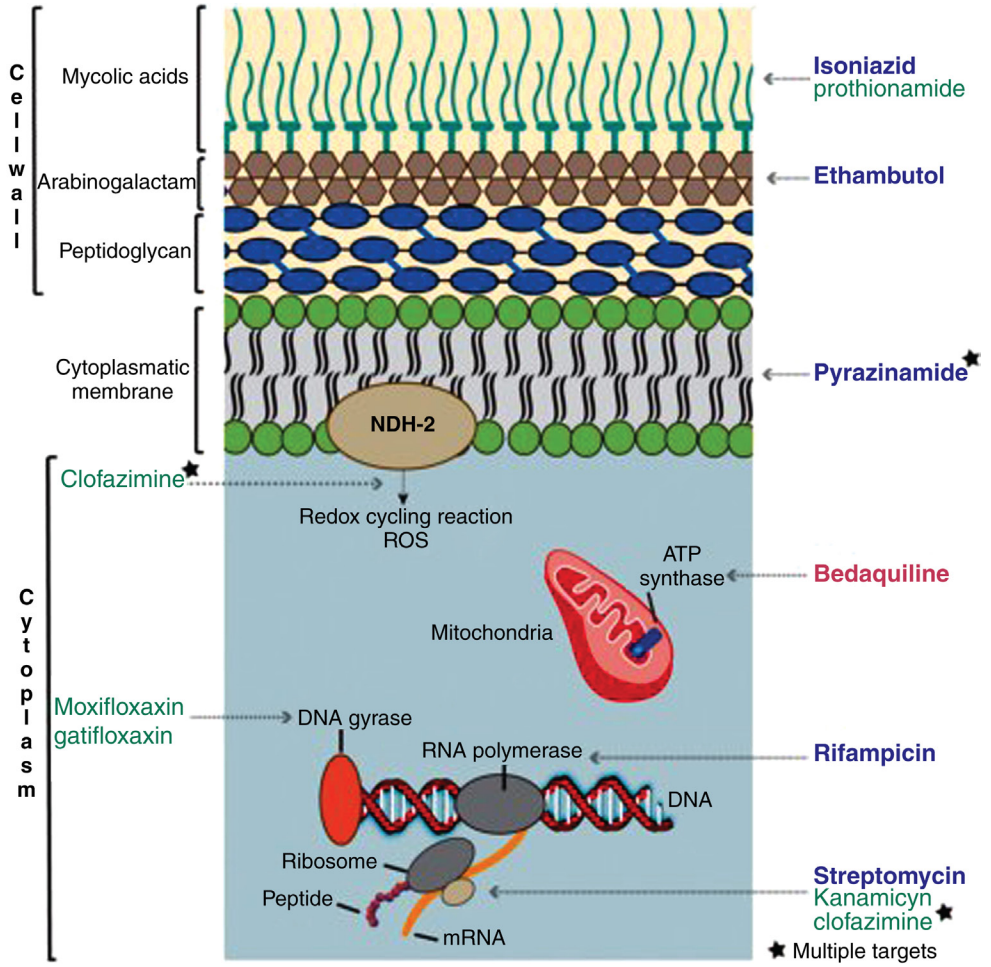

FIGURE 15.3 **Mechanism of pharmacotherapy in tuberculosis [8].**

The isoniazid's efficacy gets higher when it combined with ethambutol, rifampicin, pyrazinamide, and streptomycin. Rifampicin has the highest elimination ability of *M. tuberculosis*. Isoniazid enters bacilli by passive diffusion. The drug is not directly toxic to the bacillus but must be activated to its toxic form within the bacillus by KatG, a multifunctional catalase-peroxidase. KatG catalyzes the production from isoniazid of an isonicotinoyl radical that subsequently interacts with mycobacterial NAD and NAPD to produce a dozen adducts. One of these, a nicotinoyl-NAD isomer, inhibits the activities of enoyl acyl carrier protein reductase (InhA) and -ketoacyl acyl carrier protein synthase (KasA). Inhibition of these enzymes inhibits synthesis of mycolic acid, an essential component of the mycobacterial cell wall, leading to bacterial cell death. The products of KatG activation of INH include superoxide, H_2O_2, alkyl hydroperoxides, and the NO radical, which may also contribute to the mycobactericidal effects of INH. *M. tuberculosis* could be especially sensitive to damage from these radicals because the bacilli have a defect in the central regulator of the oxidative stress response, *oxyR*. Backup defense against radicals is provided by alkyl hydroperoxide reductase (encoded by *ahpC*), which detoxifies organic peroxides. Increased expression of *ahpC* reduces isoniazid effectiveness [10]. The antibacterial effect of isoniazid against clinical M.tb strains varies between 0.025 and 0.05 mg/L. Activity against *Mycobacterium bovis* and *M. kansasii* is moderate. Isoniazid has poor activity against MAC. It has no activity against any other microbial genus [10]. The prevalence of drug-resistant mutants is ~1 in 10^6 bacilli. TB cavities may contain as many as 10^7 to 10^9 microorganisms; preexistence resistance can be expected in pulmonary TB cavities of untreated patients. These spontaneous mutants can be selected by monotherapy; indeed, strains resistant to isoniazid will be selected and amplified by isoniazid monotherapy. Thus two or more agents are usually used. The mutations resulting in drug resistance are independent events, the probability of resistance to two antimycobacterial agents is small, ~1 in 10^{12} ($1 \times 10^6 \times 10^6$), a low probability considering the number of bacilli involved. Resistance to INH is associated with mutation or deletion of katG, overexpression of the genes for inhA (confers low-level resistance to INH and some cross-resistance to ethionamide), and ahpC and mutations in the *kasA* and *katG* genes. KatG mutants exhibit a high level of resistance to isoniazid (as shown in Fig. 15.4).

Rifampicin inhibits the growth of most Gram-positive bacteria as well as many Gram-negative microorganisms such as *Escherichia coli*, *Pseudomonas*, indole-positive and indole-negative *Proteus*, and *Klebsiella*. Rifampicin is very active against *Staphylococcus aureus* and coagulase-negative staphylococci. The drug also is highly active against *Neisseria meningitidis* and *Haemophilus influenzae*. Rifampicin inhibits the growth of many *M. tuberculosis* clinical isolates in vitro at concentrations of 0.06-0.25 mg/L [10]. The prevalence of rifampicin-resistant isolates is 1 in every 10^7 to 10^8 bacilli. Microbial resistance to rifampin is due to an alteration of the target of this drug, *rpoB*, with resistance in 86% of cases due to mutations at codons 526 and 531 of the *rpoB* gene [10].*Pyrazinamide* is the synthetic pyrazine analog of nicotinamide. Pyrazinamide is also known as pyrazinoic acid amide, pyrazine carboxylamide, and pyrazinecarboxamide. Pyrazinamide is "activated" by acidic conditions. Initially it was assumed that the acidic conditions under which pyrazinamide works were inside macrophage phagosomes. However, pyrazinamide may not be very effective within macrophages; rather, the acidic conditions for activation may be at the edges of necrotic TB cavities where inflammatory cells produce lactic acid. *M. tuberculosis* nicotinamidase or pyrazinaminidase deaminates pyrazinamide to pyrazinoic acid (POĀ), which is then transported to the extracellular milieu by an efflux pump. In an acidic extracellular milieu, a

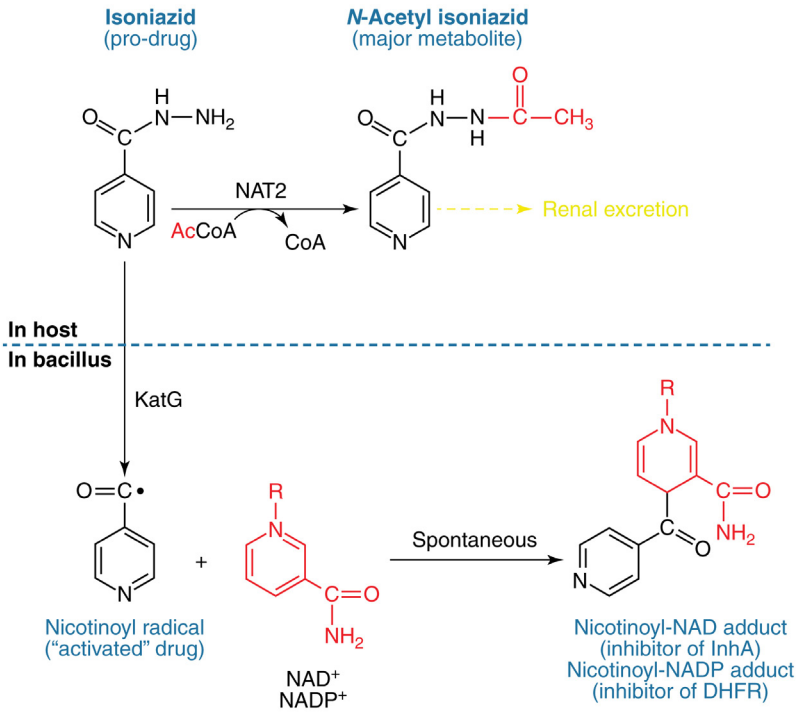

FIGURE 15.4 **Mechanism of action of isoniazid [10].**

fraction of POĀ is protonated to POAH, a more lipid-soluble form that enters the bacillus. The actual mechanism of pyrazinamide microbial kill is still unclear; three mechanisms have been proposed [10]:

1. inhibition of fatty acid synthase type I leading to interference with mycolic acid synthesis,
2. reduction of intracellular pH, and
3. disruption of membrane transport by HPOA.

Antibacterial activity of pyrazinamide in vitro only at acidic pH. At pH of 5.8-5.95, 80%-90% of clinical isolates have a MIC of 100 mg/L. Pyrazinamide-resistant occurs when M.tb has pyrazinamidase to reduce affinity for pyrazinamide. This reduced affinity decreases the conversion of pyrazinamide to POA. Single point mutations in the *pncA* gene are encountered in up to 70% of resistant clinical isolates. The mechanisms contributing to resistance in 30% of resistant clinical isolates is unclear [10].

Ethambutol inhibits arabinosyl transferase III, thereby disrupting the transfer of arabinose into arabinogalactan biosynthesis, which in turn disrupts the assembly of mycobacterial cell wall. The arabinosyl transferases are encoded by *embAB* genes. Ethambutol has activity against a wide range of mycobacteria but no activity against any other genus. Ethambutol MICs are 0.5-2 mg/L in clinical isolates of M.tb [10]. In vitro, mycobacterial resistance to the drug develops via mutations in the *embB* gene. In 30%-70% of clinical isolates that are resistant to ethambutol, mutations are encountered at codon 306 of the *embB* gene. However, mutations in this codon are also encountered in ethambutol-susceptible mycobacteria, as though this mutation is necessary, but not sufficient, to confer ethambutol resistance [10].

A combination of isoniazid-rifampicin for 9 months administration will cure 95%-98% of cases of tuberculosis infection caused by susceptible strains [9]. An initial intensive phase of treatment is recommended for the first 2 months due to the prevalence of resistant strains. The addition of pyrazinamide during this intensive phase allows the total duration of therapy to be reduced to 6 months without the loss of efficacy. In practice, therapy is usually initiated with a four-drug regimen of isoniazid, rifampicin, pyrazinamide, and ethambutol until susceptibility of the clinical isolate has been determined. In susceptible isolates, the continuation phase consists of an additional 4 months with isoniazid and rifampicin [9]. However, isoniazid and rifampicin are the TB-MDT that experiences resistant frequently.

The resistance of TB-MDT arises through several mechanisms [10] (as shown in Fig. 15.5), including:

1. The inability of TB-MDT to penetrate into *M. tuberculosis*' wall cells by reason of its rich lipopolysaccharide and mannose.
2. The anaerobic conditions in the site of infection enable *M. tuberculosis* to become dormant. The TB-MDT, especially isoniazid, is ineffective in dormant conditions.
3. Alteration of enzymes that produced by M.tb. These enzymes prevent the conversion of prodrugs into active drugs. Isoniazid inhibits the synthesis of mycolic acids, which are essential components of mycobacterial cell walls. Isoniazid is a prodrug that is activated by KatG, the mycobacterial catalase-peroxidase. The activated form of isoniazid forms a covalent complex with an acyl carrier protein (AcpM) and KasA, a beta-ketoacyl carrier protein synthetase, which blocks mycolic acid synthesis. Resistance to isoniazid is associated with mutations resulting in overexpression of

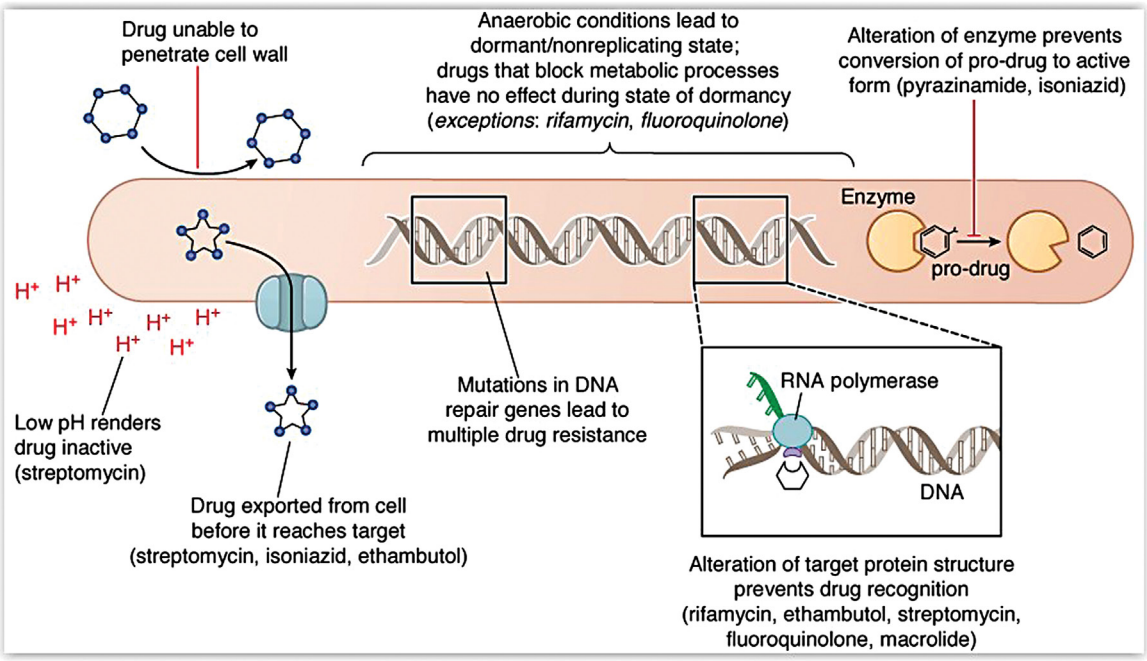

FIGURE 15.5 **Mechanism of TB-MDT resistance [10].**

inhA, which encodes an NADH-dependent acyl carrier protein reductase; mutation, or deletion of the *katG* gene; promoter mutations resulting in overexpression of *ahpC*, a gene involved in the protection of the cell from oxidative stress; and mutations in *kasA*. Overproducers of *inhA* express low-level isoniazid resistance and cross-resistance to ethionamide. *KatG* mutants express high-level isoniazid resistance and also pyrazinamide resistance.

4. Alteration of target protein structures. This prevents drug recognition (rifampicin, ethambutol, fluoroquinolones, macrolides).
5. Drug exported from the cell before reaching the sites of infection (isoniazid, ethambutol, streptomycin).
6. Mutation in DNA repair gene that leads the multidrug resistance (MDR)-TB.

Those all aforementioned mechanisms reduce the efficacy of TB-MDT.

3 Nanoparticle and its use to conquer tuberculosis infection

Nanotechnology is an innovative use of the latest technological developments or nanoparticle sized. Nowadays, it has a major impact for health and therapeutic development. The nanoparticle now is known as a drug delivery system, thus it enhances the drug efficacies [14].

In the process of diagnosis, nanotechnology may play a role in the diagnostic kit. For example in India, The aid of TB diagnosis created diagnostic kits in an optical biosensor for rapid detection of M.tb bacilli [14,15].

In the TB treatment, nanotechnology has a huge improvement in pharmacology, especially in the delivery system. The TB-MDT is able to reach the site of infection and impact to the function of macrophage and other phagocyte cells. Nanoparticle gives advantages such as combining MDT into one form, reducing the frequency of drugs, increasing the therapeutic index of anti-TB, increasing the solubility of hydrophobic agents capability, and reducing the administration of higher doses [16]. As drug delivery, nanoparticles also give other advantages such as, improve drug stability, improve the ability of carrier molecules, and the feasibility of incorporation of both hydrophilic and hydrophobic substances. Reviewing these carriers can also be designed to enable controlled (sustained) drug release from the matrix [16].

Nanoparticles as a therapeutic include; nanoemulsions, nanosuspensions, niosomes, polymeric micelles, and other self-assembled structures, are roommates antituberculosis drug nanocarriers, and polymeric and nonpolymeric nanoparticles. Nanoparticles can penetrate the intestinal permeability barrier directly through the transcellular or paracellular pathways into the circulation. This work makes drugs more and more effective. Nanosuspensions are a potential and promising new anti-TB drug formulations for intravenous way. Nanoparticles are able to achieve the higher stability and ability of the drugs, the feasibility of incorporating both hydrophobic and hydrophilic substances, and the feasibility of any administration routes such as parenteral, oral, and inhalation [15].

Nanotechnology increases the bioavailability of drugs as a result of a special absorption such as the absorption mechanism of endocytosis. The nanoparticles are also able to remain in the blood for a longer period of time and in controlled release manner into the target tissue. The self-control system of release of the drug helps reduce fluctuations in plasma and minimize side effects of the drug. In the case of TB adverse effects of tuberculosis drugs became one of the causes in poor compliance [17].

Nowadays, nanoparticle also develops in order to diminish the number of MDR-TB cases. There is a strong urge to develop novel ways of delivering the therapeutic compounds to the specific target of making the drug more effective [14].

Currently, the basic mechanism of controlled drug release was established and most drug delivery formulations were oral and transdermal administration. The effectiveness and stability were low in these drug systems. The effectiveness and stability of the drug will affect drug action and its effect on the patient's body. The drug is incorporated into the nanoparticle that easily diffused through biological membranes and cells take up. These particles for the efficiency in drug delivery to the site of action. Nanotechnology improves the performance of the effectiveness of the drug, in patients taking the drug longer, and cost-effectiveness. Nanotechnology can produce biodegradable, biocompatible polymers, stimulate, and targeted by following the intended target organs, such as liposomes deliver responsive, nanofabricated materials (fullerenes, carbon nanotubes, silicon, silica), metals (gold, silver, iron, platinum, quantum dots), and polymers (micelles, dendrimers). Nanoparticles shape assortment such as spherical, rods, wires, discs, hemispherical, and ellipsoidal [17].

The size of the nanoparticles that less than submicron (<1 μm) colloidal particles are used as drug delivery vehicles. For therapeutic purposes, drugs can be covalently embedded to the particle surface or can be incorporated in the matrix of the particle. Nanoparticles comprise biocompatible and biodegradable materials such as polymers, which can be natural (e.g., gelatin and albumin), synthetic (e.g., polylactides and polyalkylcyanoacrylates), or solid lipids. Nanoparticles have a higher efficiency of the cells compared to the molecules in the case of a delivery system. Nanoparticle delivery system has capabilities that are more specific and faster. These carriers that are adapted to enable controlled, slow, and persistent drug release from the matrix [18]. The nanoparticle expresses in the gene and able to trace into the DNA complexes track (in vivo), this advantage is important in simultaneously dosage administration and determination. In this determination system, the nanoparticle has a high sensitivity to measure the level of gene expression (in vivo imaging) and is also able to target the specific/diseased cell types [14].

Nanoscience is a new perspective in making early detection, prevention, diagnosis, and treatment in TB became easier and more effective. This is because nanoscience has the potential to empower local responses to specific targets and other benefits to save costs. Nanoparticle-based gene therapy and drug delivery hold a great promise for the sound management of diseases in terms of improved drug bioavailability and reduced dosing frequency, though it is extremely important to investigate the toxic effects nanoparticles according to chemistry, size, and other physical properties [14].

Future holds up in designing of drug-delivery systems or formulations roommates can resolve all the limitations of tuberculosis drug therapy and making them affordable to all patients. Several antitubercular drugs encapsulated in natural or synthetic carrier-based controlled release formulations have been explored and nanoparticles appeared to be the best in terms of therapeutic efficacy [14].

Developing research related to vector-based delivery systems could combine roommates colloidal carriers such as large payloads of drug with the active targeting to improve the effectiveness and efficiency of drug action based on the nanoparticle-based formulations. Understanding the fate of nanocarriers and their polymeric constituents along with the elimination of any residual organic solvents is a must for dealing with any toxicological issues associated with these nanoformulations [14].

Nanoparticle delivery system is a promising key for the media but it is also against drug-susceptible tuberculosis and drug resistance. Nanoparticles are also useful for reducing the burden on the patient's dose but have the same benefits, but simultaneously. Nanotechnology still has a lot of homework for future challenges especially for TB. Lots of health gaps need to be filled along with global sustained efforts to

overcome TB infection, in order to reach the site of infection in the secondary lymphoid organs [14].

Increasing incidence of multidrug-resistant strains make research related to ways of delivering the therapeutic compounds to the specific target of growing. Many delivery systems, such as nanoemulsions, nanosuspensions, polymeric and nonpolymeric particles, liposomes, niosomes, and dendrimers, have been developed in the past, overcoming many of the shortcomings of the delivery of conventional drugs [14].

4 Function nanoparticle for overcoming resistance tuberculosis treatment

TB drug resistance is the inability of the existing TB drugs to phagocyte mycobacterium that exists in the patient's body, due to the growing strength of *Mycobacterium tuberculosis* that is inside the patient's body TB [19].

Mycobacterium tuberculosis is one of the most infection disease which successful human pathogens, due to its ability to carry a primary infection to a state of dormancy (latents), persisting in the body even in immune-competent people. In this regard, it is important to mention that there are two billion people infected worldwide by M.tb, and only nine million people develop into TB clinical diseases, for example: from 100 people whom were exposed with M.tb, only 2 persons grow into clinical TB infection, this phenomenon is due to immune response. TB is usually a lung disease, due to the fact that these organs are the gateway and provide optimal conditions for the infected of this disease. The primary infection begins with the inhalation of the particles of *Mycobacterium tuberculosis*. Approximately 10% of this invasion due to respiratory tract that are in alveoli and bronchioles, where the bacteria is recognized and phagocytozed by alveolar macrophages (AMs) or dendritic cells (DCs). Macrophages exposed to *Mycobacterium tuberculosis* secrete pro-inflammatory cytokines (IL-1, TNF-α, and IL-6) that will contribute to the subsequent formation of focal granulomatous lesions, a process that takes 2-3 weeks, and which generally leads to the containment of the pathogen [8].

Pharmacotherapy of tuberculosis therapeutic regimen is recommended by the WHO for susceptible pulmonary TB rifampicin, isoniazid, pyrazinamide, ethambutol, and streptomycin. The therapy in tuberculosis consists of a short-term treatment of 6 months (divided into two stages), intensive phage and continue phage [8,10,20]. Drug chemical structures could be seen in Fig. 15.6.

Tuberculosis drug resistance or multidrug resistant tuberculosis (MDR TB) is a condition in which a patient is resistant to first-line TB treatment. The first-line drug is a list of the first drug given to patients with TB occurrence. This is because of the resistance of patients who dropped out treatment or in patients who are infected with *Mycobacterium tuberculosis* for the umpteenth time. The inability of antituberculosis (anti-TB) has become one of the causes in which a patient MDR-TB or now known by extra-drug resistant tuberculosis (XDR-TB) must increase the dosage of antituberculosis drugs and make the treatment of TB becomes longer. The efficacy of antiinfective drugs is not only dependent on the pathogens related to MIC, but also on the exposure of the drug in the patient [21].

Combination of nanomaterials with the understanding of differentiation of biological processes, nanotechnology could ameliorate and trigger the usage of brand new drug/antigen in delivery systems. Based on evidence nanomaterials have a result better than liposomes, there about stabilization and drug loading capacity. The differentiation of nanoformulations, like lipid-based and (branched) polymeric ones, is being explored to deliver different types of drugs. In recent years, many efforts have been directed to the encapsulation of anti-TB drugs within nanoparticles [8].

Low compliance, the main cause that makes the incidence of drug resistance to anti-TB

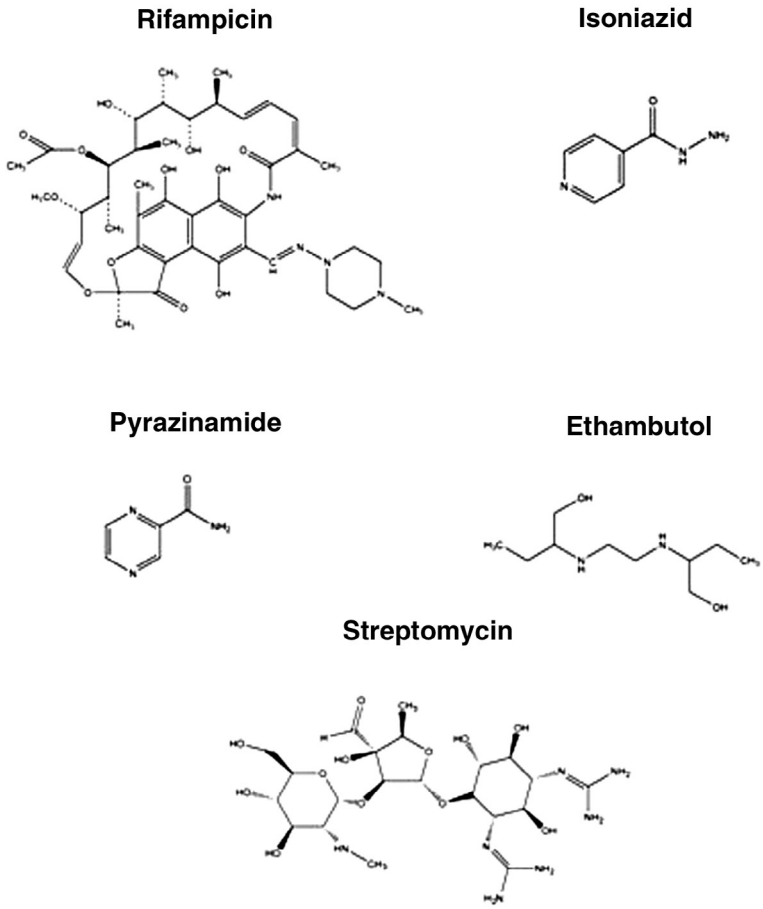

FIGURE 15.6 **Pharmacotherapy of tuberculosis** [10].

against *Mycobacterium tuberculosis*, is increasing. Now it has become clear that pharmacokinetic variability is much more likely to be the driving force of drug resistance. The development of pharmacokinetic required in the case of antituberculosis drug resistance (anti-TB). If the existing infection with TB addressed adequately, then the bacteria will not occur and can be treated. However, in the case of anti-TB drugs, a therapeutic range or the target has not been established.[21].

Nanoparticles are believed to increase the significance of the treatment of TB from diagnosis, treatment, and prevention. Nanotechnology is one of the functions that improve nanomedicine. Due to reviews, their size, shape, and morphology (less than 100 nm in one aspect) nanoparticles exhibit different properties of the same material when they are in bulk size. New diagnostics and therapeutics for application in organ systems have been developed due to the unique properties of nanopharmaceuticals [22].

Nanomedicine approaches are being used as effective carriers of drugs to different parts of the body that were previously difficult to access. Nanomedicine approach in anti TB enhances the efficacy of the drugs. It may possible to the target nanoparticles to specific organs by modifying

the elemental composition, size, shape, charge, and surface modification or functionalization [22].

That brings us to the second reason why we need new anti-TB drugs. Drug resistance has emerged as a phantom from the dark, threatening today every corner of the world. Often RIF-resistance correlates to the MDR category (resistant to INH and RIF). XDR is an MDR *M. tuberculosis* strains resistant to any fluoroquinolone also and at least one injectable agent. The prognosis is less favorable for harboring XDR-bacilli patients compared to patients with MDR, with a five times higher risk of death, therefore the XDR patients need to be hospitalized or requires longer treatment times. However, it has been shown that within an aggressive treatment, XDR-TB patients have been successfully cured by 60%. The treatment of M/XDR-TB usually takes more than 2 years, and requires the use of more toxic, less effective, and more expensive drugs [15].

Pulmonary tuberculosis is the most ubiquitous form of the disease, and the respiratory path represents the means of delivering a unique ATD's directly to the lungs. The reduction of toxicity and accomplishing higher systemic drug concentration at the chief site of infection are the promising advantages of the direct delivery of the drug to the lungs. Inhalable nanoparticles possess an enhanced ability of mucosal adherence, particle delivery, and net drug delivery to the lungs [23].

Anti-TB drug carriers are classified: synthetic or natural origin. They allow the flexibility of selecting the route of drug delivery, Depending on the drug formulation. Not only the smaller size but also the ability of higher drug encapsulation and enhancement of the orally administered-drug bioavailability is the key difference between the nanoparticles and microparticles. poly-DL-lactide-co-glycolide (PLG)-A nanoparticles are commonly used in preparation for emulsification or evaporation [15].

Nanomedicine approach significantly prolonged provided a mean residence time, and elimination half-life of the drugs in comparison to the conventional orally administered formulations and resulted in an enhanced relative bioavailability for the nanoparticle-preparations (rifampicin, pyrazinamide, and isoniazid). The nebulization of the nano-encapsulated drug led to an absence of *Mycobacterium tuberculosis* in the lungs [17].

Drug resistance tuberculosis is an important health issue in progress made in TB care and control programs worldwide. Drug resistance arises due to pathogen inappropriate use of medicines in the treatment of drug-susceptible tuberculosis patients. This improper use may be due to the administration of improper treatment regimens and the failure of noncompliance of the patients to complete the course of therapy [17].

The treatment of tuberculosis has become a challenge for the physicians because of the emerging threat of drug-resistant strains of the pathogen. The *M. tuberculosis* responsible for the disease can overcome the cellular defense mechanisms, infecting the cells and turning them into reservoirs. The drawbacks of conventional tuberculosis drug formulation are the inability to kill the intracellular pathogens because of reviewing their limited bioavailability and limited penetration power in the targeted pathogens to produce a therapeutic effect [17].

Nanomedicine has the potential to challenge such limitations and improve the therapeutic efficacy of such drugs. Nanovesicles formulation of gentamicin, vancomycin amikacin, kanamycin, streptomycin, present enhanced in vitro and in vivo efficacy. Reviewing these formulations successfully reduced the viable bacteria counts of *M. tuberculosis*. However, in some cases, the pulmonary availability of the drugs was small or absent roommates can overcome by the development of localized particles targeted for delivery by inhalation or by targeting the pulmonary area [17].

5 Nanoparticle for diagnose tuberculosis

Diagnose tuberculosis with detection of mycobacterium DNA in clinical samples using nanoparticles has been developed, and it is a great futuristic vision. Nanocrystalline silicon photodetector with suitable software can detecting tuberculosis for diagnose tuberculosis and it could be to lessen the human error in diagnose tuberculosis. Recently, a convenient and low-cost biosensing platform was presented to detect *Mycobacterium tuberculosis* [24].

Exploated of scanobased, fluoremetric, colorimetric, surface-enhanced Raman scattering and electrochemical methodologies as a ultrasensitive techniques can developed to detect gold nanoparticles in clinical sputum samples, which that engineered with thiol-modified oligonucleotides to make the detection efficient, simplified, and relatively cheaper. *Mycobacterium tuberculosis* can be easily differentiated from other members of Mycobacterium species with the help of these nanoparticles. Fluorescent semiconductor quantum dots and magnetic beads are also used to detect DNA of Mycobacterium species without prior PCR. Even the probe consisting of superparamagnetic ironoxide nanoparticles has been designed and this probe is specific for diagnosing the extrapulmonary TB [24].

Nanoparticles are versatile and diverse with respect to their properties and structural, which enables them to be used for clinical diagnosis and effective drug delivery purpose in a unique and more reliable manner. Owing to their wide range of distribution manner and functional modes, nanoparticles can be used for multiple applications. The main reason behind this is the excellent geometric control of structures by arresting their formation during different stages of their synthesis. Better and more effective disease treatment protocols can be achieved with the use of systems like programmable nanorobots that can be employed for site-specific drug delivery [24].

Dendrimers has a layer, outer core on dendrimers there has hydrophilic characteristic and on an inside core on dendrimers has hydrophobic characteristic, so possible to deliver material component (as shown in Figs. 15.6 and 15.7). This arrangement forms the basic formulation

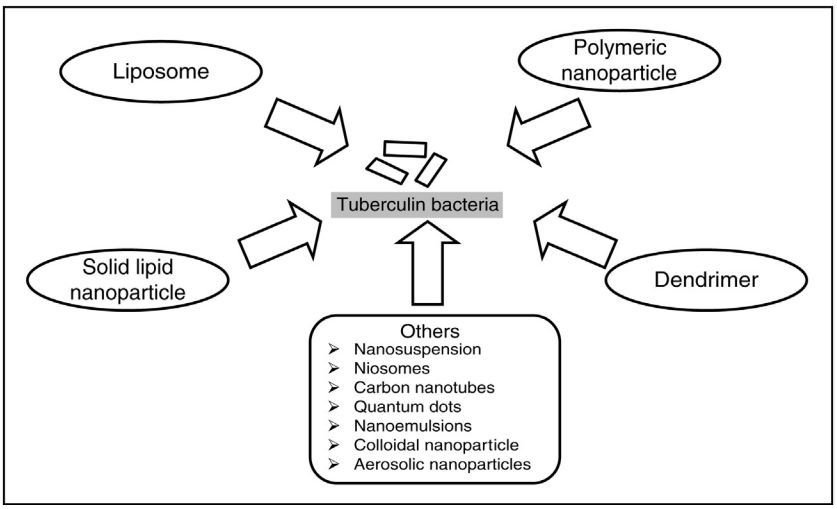

FIGURE 15.7 **Different nanotechnology-based approaches [8].**

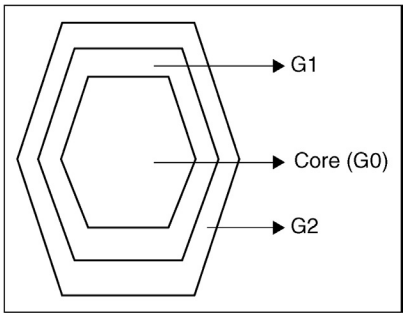

FIGURE 15.8 Dendrimers based nanotechnological treatment [8].

for dendrimers in drug delivery systems. Mycobacterium is a Gram-negative bacterium. Its cell-wall composition (a rich layer of mycolic acid) renders it difficult for potential anti-TB medicinal preparations to enter into the infected cells. With the use of dendrimers, the conformation of the carried medicinal formulation is biochemically altered in a way that favors its entry into the specific target cells [24].

Nanoparticles have also been explored for the coencapsulation of tuberculosis drugs. Respiratory delivery developed because nanocarriers loaded with RIF, INH, and PYR. Data demonstrated that the inhalable nanotechnological platform allowed improving the pharmacotherapy regimen in *Mycobacterium tuberculosis* infected. Nanoparticles were investigated to encapsulate these tuberculosis drugs. Polymeric micelles (PMs) have become well-investigated nanovehicles. They are composed by amphiphilic biocompatible polymers that can self-assemble into nanostructures when the polymers concentration is above their critical micellar concentration. The pharmacotherapy of tuberculosis: this strategy has not been explored as LPs and NPs. The selection of the biomaterials and the pharmaceutical additives allowed developing different dosage forms (Figs. 15.8 and 15.9) [8].

Nanosuspension can make more efficient absorption and better biodistribution of drug molecules. During the formulation of a nanosuspension, the crystalline particles of the drug are converted into amorphous form. The conversion to amorphous forms can be achieved using X-ray diffraction. Various parameters such as particle size, charge distribution, and drug dissolution celerity also can be more effectively and easily monitored as well as suitably modified to suit a particular kind of drug delivery mechanism. Nanoemulsions represent a stable thermodynamic mixture. The use of rifampicin-based nanoemulsions for TB pharmacotherapy: they have elaborated the critical design features such as viscosity, solubility, and chemical interaction ability for nanoemulsion design to become optimized drug delivery vehicles. It has been successfully used for the killing of *Mycobacterium tuberculosis* germs at low dosage, and there is hardly any risk of toxicity or side effects (Fig. 15.10) [24].

There is a significant improvement from anti-TB drugs with nanoparticle than free anti-TB drugs. Relativity, bioavailability and mean residence time of encapsulated drugs more significant. Five aerosolized doses of PLG nanoparticles coencapsulating rifampicin, isoniazid, and pyrazinamide revealed undetectable cfu in the lungs. Comparison with microparticles: first, the decrease of lung cfu was better, and second, coadministration of three anti-TB drug encapsulations was possible in nanoparticles delivery system. As detection of *Mycobacterium tuberculosis* nanotechnology more expendable and efficient, especially high sensitivity so diagnose of tuberculosis more effective and efficient because nanoparticles can be tagged with suitable ligands and can be functionalized with various lectins to make more effective PLG nanoparticle uptake [15].

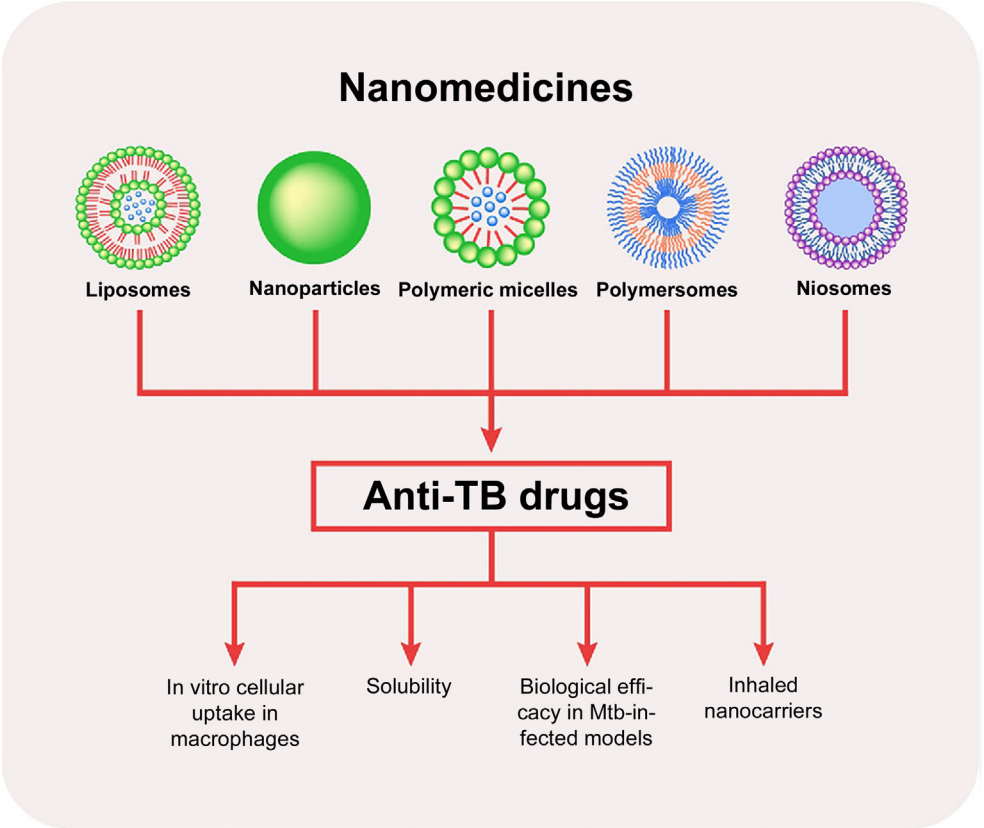

FIGURE 15.9 **Nanomedicine in tuberculosis [8].**

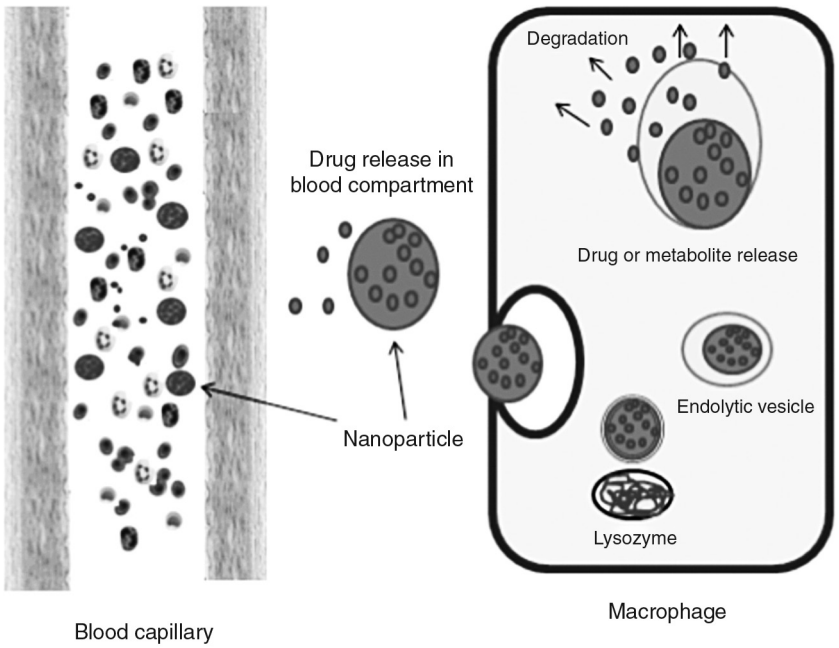

FIGURE 15.10 **Mechanism of both natural and synthetic drug carriers by nanoparticle [14].**

References

[1] A.K. Abbas, A.H. Lichtman, S. Pillai, Cellular and Molecular Immunology, sixth ed. Saunders, Philadelphia, 2012.

[2] E. Campion, Latent Mycobacterium tuberculosis infection Haileyesus, N. Engl. J. Med. 370 (22) (2015) 2127–2135.

[3] B.I. Restrepo, L.S. Schlesinger, Host-pathogen interactions in tuberculosis patients with type 2 diabetes mellitus, Tuberculosis 93 (2013) S10–S14, doi: 10.1016/S1472-9792(13)70004-0.

[4] S.S. Stew, P.J. Martinez, L.S. Schlesinger, B.I. Restrepo, Differential expression of monocyte surface markers among TB patients with diabetes co-morbidity, Tuberculosis. 93 (2013) S78–S82, doi: 10.1016/S1472-9792(13)70015-5.

[5] Pusat Data dan Informasi Kementerian Kesehatan RI, Tuberkulosis.Kementerian Kesehatan RI, Jakarta, 2018.

[6] World Health OrganizationGuidelines for surveillance of Drug Resistance in Tuberculosis, fifth ed., WHO Publications, (2015).

[7] E. Grotz, N. Tateosian, N. Amiano, M. Cagel, E. Bernabeu, D.A. Chiappetta, et al. Nanotechnology in tuberculosis: state of the art and the challenges ahead, Pharm. Res. 35 (2018) 213.

[8] B.G. Katzung, Basic & Clinical Pharmacology, fourteenth ed., McGraw-Hill Education, San Francisco, (2018).

[9] B. Laurence, B. Chapner, B. Knollmann, The Pharmacological Basis of Therapeutics-Goodman & Gillman-Ed, twelfth ed., McGraw-Hill Companies, California, (2011).

[10] A. Manuscript, C. Death, Cell death and autophagy in TB Andrew, Semin. Immunol. 26 (6) (2015) 497–511.

[11] B.D. Novita, Metformin: a review of its potential as enhancer for anti tuberculosis efficacy in diabetes mellitus-tuberculosis coinfection patients, Indian J. Tuberc. 66 (2) (2019) 294–298, doi: 10.1016/j.ijtb.2019.02.013.

[12] D. Golan, A. Tahjian, E. Armstrong, A. Armstrong, Principles of pharmacology: the pathophysiologic basis of drug therapy, third ed., Lippincott Williams & Wilkins, Philadelphia, (2012).

[13] A.K. Sharma, R. Kumar, B. Nishal, Nanocarriers as promising drug vehicles for the management of tuberculosis nanocarriers as promising drug vehicles for the management of tuberculosis, Bionanoscience 3 (June) (2013) 102–111.

[14] A. Cheepsattayakorn, C. Mai, Roles of nanotechnology in diagnosis and treatment of tuberculosis, J. Nanotechnol. Diagn. Treat. 1 (May) (2013) 19–25.

[15] J.P. Mathuria, Nanoparticles in tuberculosis diagnosis, treatment and prevention: a hope for future, Digest J. Nanomater. Biostruct. 4 (2) (2009) 309–312.

[16] J. Wanigasekara, C. Witharana, Applications of nanotechnology in drug delivery and design-an insight, Curr. Trends Biotechnol. Pharm. 1 (January) (2017) 78–91.

[17] M. Nasiruddin, K. Neyaz, S. Das, Nanotechnology-based approach in tuberculosis treatment, Tuberc. Res. Treat. 2017 (2017) 1–12.

[18] WHO, Guidelines for Surveilance of Drug Resistance in TBC, fifth ed., Switzerland, (2015) p. 76.

[19] D. Resistance, T. Azimi, M. Mosadegh, M.J. Nasiri, S. Sabour, S. Karimaei, et al. Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review, Infect. Drug Resist. 12 (2019) 2943–2959.

[20] P.C. Tan, G. Rajasingam, S. Devi, S.Z. Omar, Dengue infection in pregnancy: prevalence, vertical transmission, and pregnancy outcome, Obstet. Gynecol. 111 (5) (2008) 1111–1117.

[21] J.D. Guzman, X. Montes-rincón, Research and development of new drugs against tuberculosis, in: Tuberculosis-Current Issues in Diagnosis and Management, Intech (2014) 331–358.

[22] S. Banyal, P. Malik, H.S. Tuli, T.K. Mukherjee, Advances in nanotechnology for diagnosis and treatment of tuberculosis, Wolters Kluwer Heal. 19 (3) (2013) 289–297.

Index

Note: Page numbers followed by "f" indicate figures.

W

X

Nanotechnology Based Approaches for Tuberculosis Treatment

Edited by
Prashant Kesharwani
Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard (A Government-aided Deemed University), New Delhi, India

Nanotechnology Based Approaches for Tuberculosis Treatment discusses multiple nanotechnology-based approaches which may help overcome persisting limitations of conventional and traditional treatment of tuberculosis (TB). A quarter of the world's population is infected with TB, and despite potentially curative pharmacotherapies being available for over 50 years, TB deaths persist, and multidrug-resistant strains of TB are emerging due to prolonged treatment, high pill burden, and low patient compliance. The book summarizes the pathogenesis, biology, immunology, drug regimens and multidrug resistance of tuberculosis followed by various types of nano drugs, their synthesis, formulation, characterization, and applications, along with the most important administration routes. It also explores recent advances and achievements regarding therapeutic efficacy and provides possible future applications in this field. *Nanotechnology Based Approaches for Tuberculosis Treatment* directly addresses translational aspects and clinical perspectives of tuberculosis nanomedicine from a comprehensive and multidisciplinary perspective. It will be a useful resource for investigators, pharmaceutical researchers, innovators, and scientists working on technology advancement in the areas of designing targeted therapies, nano scale imaging systems and diagnostic modalities in tuberculosis. *Nanotechnology Based Approaches for Tuberculosis Treatment* will also cater to the basic needs of students and new researchers in the fields of tuberculosis and nanomedicine.

Key Features

- Addresses the gap between nanomedicine late discovery and early development of tuberculosis therapeutics
- Explores tuberculosis nanomedicine standardization and characterization with newly developed treatment, diagnostic and treatment monitoring modalities
- Covers the field thoroughly from pathogenesis of tuberculosis and multi-drug resistant mycobacterium tuberculosis to treatment approaches using nanotechnology and different nanocarriers

ACADEMIC PRESS
An imprint of Elsevier
elsevier.com/books-and-journals