REVIEW PAPER

Advances in Targeted Pyrazinamide Delivery via Functionalized Chitosan Nanoparticles

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ABSTRACT

Mycobacterium tuberculosis causes tuberculosis (TB), which continues to be a serious health risk in the world; there are around 10 million cases of TB each year and about 1.2 million deaths. Pyrazinamide (PZA) is a first line anti tubercular drug which is highly bactericidal against the intracellular mycobacteria especially in acidic environment. Its clinical efficacy is, however, hampered by low bioavailability, insufficient intracellular uptake, systemic toxicity and the increasing problem of drug resistance. Chitosan nanoparticles are becoming a promising drug delivery system because of the following properties; biocompatible, biodegradable, cationic and mucoadhesive properties. The review aims to provide a comprehensive overview of the progress in targeted PZA delivery systems based on functionalized chitosan nanoparticles, condensing the existing information from 2014 to 2024. Chemistry of chitosan, methods of synthesis of nanoparticles, targeting the ligands, cellular uptake mechanism and translation of the nanoparticles from preclinical to clinical settings are discussed. Moreover, the synergic effect of combination therapies, the challenges in the intracellular delivery, and future prospects for overcoming drug resistance and enhancing therapeutic efficacy are discussed. This review aims to give insights to the development of good nanoparticulate formulations for improving the anti-tuberculous treatment.

Keywords: Tuberculosis, Intracellular delivery, Functionalized nanoparticles, Pyrazinamide, Targeted drug delivery, Chitosan nanoparticles

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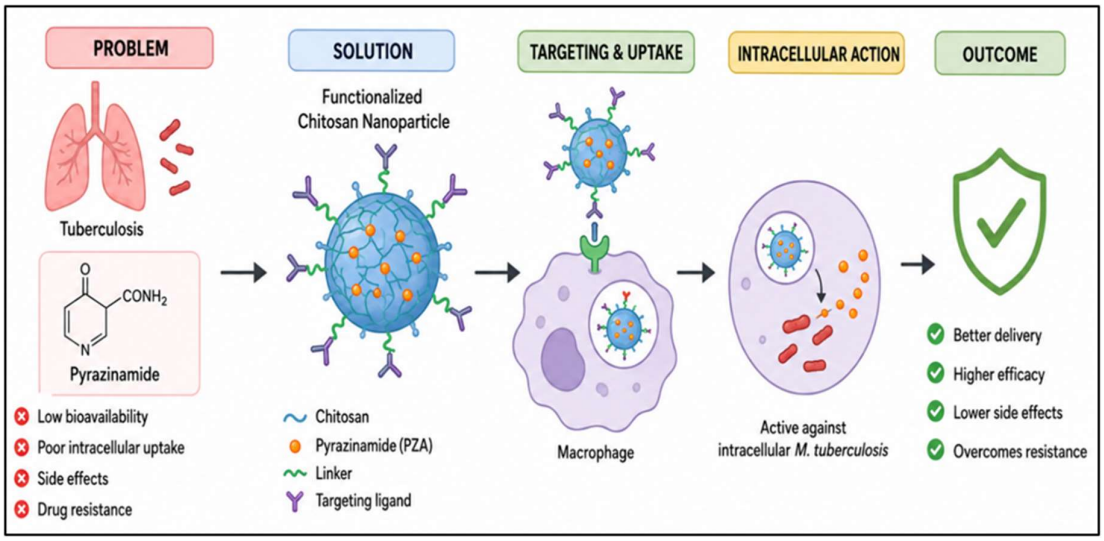

Figure 1: Schematic Illustration of Targeted Pyrazinamide Delivery via Functionalized Chitosan Nanoparticles Against Mycobacterium tuberculosis

1. INTRODUCTION

The TB disease is one of the most devastating diseases in the world. The World Health Organization (WHO) estimates that there were 10.6 million TB cases in 2021 and an estimated 1.4 million TB-related deaths [1]. It occurs mainly in the lungs, but can also affect the body's other organs, leading to serious complications. Treatment regimens have become more complex and the cure rate has been lowered by the emergence of multi drug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains [2].

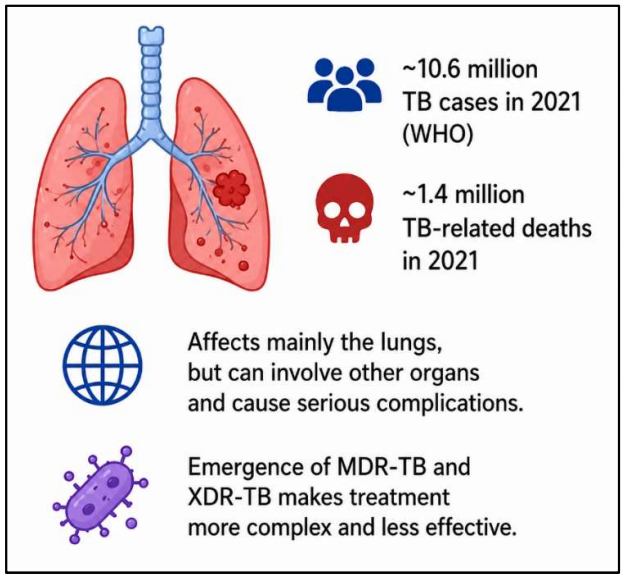

Figure 3: Challenges and Limitations of Pyrazinamide in Tuberculosis Therapy

Pyrazinamide (Pyrazine-2-carboxylic acid amide, PZA) is an approved first-line anti-tuberculous drug by the FDA used to treat TB that is a prodrug [3]. M. tuberculosis has the ability to convert it to its active metabolite pyrazinoic acid (POA) through bacterial pyrazinamidase activity that disrupts the proton-motive force across the mycobacterial cell membrane [4]. However, PZA has some drawbacks:

(i) poor aqueous solubility and extensive first-pass metabolism, resulting in low oral bioavailability
(ii) high potential for resistance development
(iii) low ability to cross the macrophage membrane efficiently for delivery to intracellular pathogens
(iv) narrow therapeutic window with hepatotoxicity and hyperuricemia [5].

Table 1: Major Drawbacks and Limitations of Pyrazinamide (PZA) [6]

S. No.	Drawback of PZA	Description
1	Poor aqueous solubility and extensive first-pass metabolism	Results in low oral bioavailability
2	High potential for resistance development	Increases the likelihood of drug-resistant infections
3	Low ability to cross the macrophage membrane efficiently	Limits delivery to intracellular pathogens
4	Narrow therapeutic window	Associated with hepatotoxicity and hyperuricemia

Targeted delivery of pharmaceuticals to disease sites via nanotechnology-based drug delivery systems has revolutionized therapeutic approaches, allowing for minimal side effects while delivering maximum efficiency of drug. Nanotechnology-based drug delivery systems have revolutionized therapeutic approaches by allowing for the delivery of pharmaceuticals to disease sites with minimal side effects and maximum efficiency [7]. Chitosan-based nanoparticles have attracted a great deal of interest because of their outstanding properties such as biocompatibility, biodegradability, low toxicity and the absence of immunogenicity [8]. A positively-charged biopolymer, chitosan, is produced from chitin and exhibits inherent mucoadhesive and permeation-enhancing properties, which enhance cell uptake [9]. The functionalization of chitosan nanoparticles with specific ligands (such as antibodies, peptides, carbohydrates) facilitates targeted delivery to specific cell types involved in disease, improving therapeutic potential and decreasing off-target effects in the body [10].

This is a comprehensive review that focuses on recent developments in targeted delivery of PZA through functionalized chitosan nanoparticles. Synthesis techniques, characterization, targeting, cellular uptake mechanisms and preclinical to clinical considerations are explored. A particular focus is on barriers to intracellular delivery, combination therapies, and approaches to deal with acquired drug resistance.

2. EPIDEMIOLOGY AND CURRENT CHALLENGES OF TB.

2.1 Epidemiology and Public Health Impact

TB remains a disease of the past, and it is still a major public health threat all over the world [1]. It is a disease caused by bacterium M. tuberculosis and mainly attacks the lungs, but may involve any organ system. According to the latest Global TB Report by the WHO, TB incidence rates have been decreasing by around 2% per year over the last decade, but this is not enough to reach the SDG targets [1]. The distribution of disease burden is also not uniform across the

world, and around 87% of all TB cases in the world occur in the Southeast Asian, African and Eastern Mediterranean regions [2]. In fact, almost 45% of the TB burden in the world is borne by India, China and Indonesia combined [3]. The transmission of TB is via respiratory droplets from an infected person's coughing, sneezing or talking [4]. Malnutrition, human immunodeficiency virus (HIV) co-infection, diabetes mellitus, silicosis and immunosuppression are all risk factors for TB infection and progression [5]. Of particular interest is the fact that HIV/TB co-infection represents clinical challenges with TB being the leading opportunistic infection in people living with HIV/AIDS [6].

2.2 Limitations of Current Anti-Tuberculous Therapy

Treatment for TB currently consists of a standard course of medication: isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) for 2 months followed by isoniazid (INH) and rifampicin (RIF) for 4 months [7]. This therapy has helped to substantially increase the cure rate of TB, but there are several difficulties with this therapy, including:

(i) extended treatment duration causing poor adherence to treatment
(ii) many side effects of the drugs
(iii) drug interactions
(iv) the emergence of drug-resistant strains
(v) drugs are not well penetrated to the site of infection, such as in macrophages and caseous lesions [8].

TB has become a serious problem of drug resistance to the global TB control programme [9]. MDR-TB is a strain that is resistant to INH and RIF, the two most effective anti-TB drugs, whereas XDR-TB is a strain that is also resistant to fluoroquinolones and second-line injectable drugs [10]. Globally, MDR-TB is estimated to be responsible for around 3.3% of TB cases, and is more prevalent in the Eastern European and Central Asia region [2]. MDR-TB requires longer treatment (20-24 months) using multiple drugs, higher treatment cost and lower cure rates (55-65%) than drug-susceptible TB (85-90%) [11].

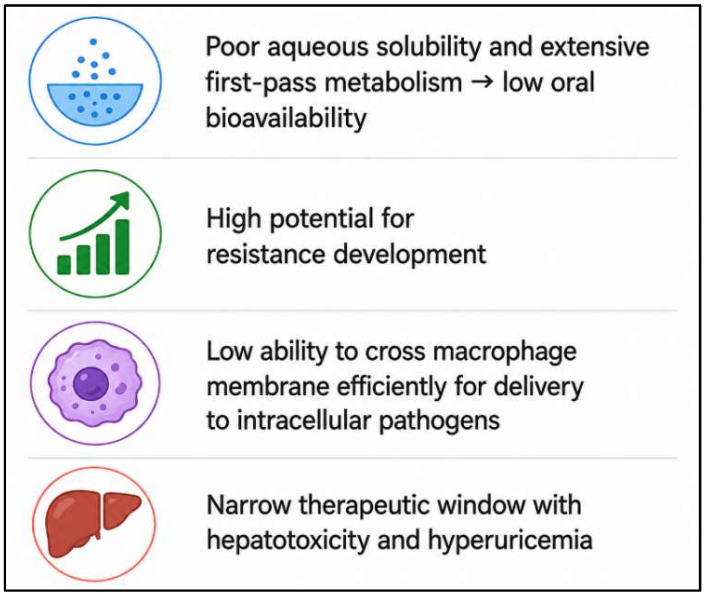

Figure 2: Major Limitations Associated withPZA Therapy in Tuberculosis Treatment

3. PYRAZINAMIDE: PHARMACOLOGY, MECHANISM OF ACTION, AND LIMITATIONS

3.1 Chemistry of Pyrazinamide and its structure

Pyrazinamide (Figure 1) is a synthetic analog of nicotinamide, and has the molecular formula C5H4N2O and a molecular weight of 123.11 g/mol [12]. It is a weak acid with a pKa of 0.5 and another pKa of 9.2 and therefore is not stable in acidic environment as free acid [13]. Due to its low solubility in water at physiological pH (ca. 5 mg/mL) and low lipophilicity (LogP = 0.59), low membrane permeability and bioavailability [14] are expected. PZA is a zwitterion at physiological pH which accounts for its poor cellular uptake [15].

3.2 Mechanism of Action

Pyrazinamide is a prodrug which needs enzymatic activation in bacteria to be effective against TB [16]. The activation mechanism is as follows:

(i) PZA is converted to pyrazinoic acid (POA) by the bacterial enzyme pyrazinamidase (PncA) encoded by pncA gene [17]
(ii) POA is then converted to pyrazinylthionicotinamide (PTNH) via a two-step process involving acyl-CoA synthetase
(iii) PTNH inhibits the essential enzyme in the NAD+ salvage pathway, namely NADH-dependent NAD+ synthetase [18]. This inhibition results in a reduction of the proton-motive force across the

mycobacterial cell membrane and depletion of NAD+ [19]. Consequently, ATP production is inhibited and the bacterial cell can no longer use this ATP-dependent process required for its survival, resulting in death of mycobacterial cells [20].

One of the special properties of PZA is its activity against persistent mycobacteria and its bactericidal activity in an acid pH as in activated macrophages and acidic environment of TB granulomas [21]. This property is important in combination therapy and to treat intracellular mycobacteria [22].

3.3 Clinical Limitations and Resistance Mechanisms

Although effective, there are several reasons why PZA may be clinically limited [23]. Its narrow therapeutic window makes optimization difficult, as high doses will cause hepatotoxicity and hyperuricemia [24]. POA accumulation causes hepatotoxicity and hyperuricemia is caused by PZA's interference with renal urate excretion by competitive inhibition at URAT1, the urate transporter in the kidneys [25]. L liver injury, mainly from PZA, occurs in about 1-3% of TB patients, with more people having underlying liver disease or co-infection with HIV [26].

Second, the low aqueous solubility of PZA at physiological pH, rapid first-pass hepatic metabolism mediated by xanthine oxidase, incomplete gastrointestinal absorption and variable plasma concentrations among patients are responsible for the low oral bioavailability [27]. The result of these factors is that an intracellular level of the drug at the site of infection becomes sub-optimal [28].

Third, mutations in pncA gene, which results in the production of pyrazinamidase, cause PZA-resistant TB. [29] The mutations that occur in pncA are responsible for 72-97% of PZA resistant isolates and cause PZA inactivation [30]. In addition, resistance may occur due to mutation of the rpsA gene (ribosomal protein S1) and other genes involved in PZA metabolism [31]. PZA-resistant TB is a substantial treatment challenge due to the need for a change in regimen with fluoroquinolones which have less efficacy and have a longer duration of treatment [32].

4. CHITOSAN-BASED NANOPARTICLES: CHEMISTRY AND SYNTHESIS

4.1 Chitosan Chemistry and Properties

Chitosan is a linear biopolymer of D-glucosamine, a derivative of D-glucose containing units of 2-amino-2-deoxy-D-glucopyranose (D-glucosamine) linked together by β-(1,4) bonds [33]. It is a natural biopolymer found in crustacean shells, insects and fungi, and is synthesized via partial deacetylation of chitin [34]. Generally it is between 75%-100% deacetylation (DD), which indicates the number of free amino groups available for chemical modification [35]. The amino groups impart chitosan with a pKa value of around 6.5, which renders chitosan a weak polyelectrolyte that is positively charged at physiological pH [36].

Cationic nature, biocompatibility, biodegradability, mucoadhesivity and intrinsic immunomodulatory properties are characteristics of chitosan that make this molecule particularly useful for drug administration:

(i) biocompatible as it is not toxic to mammalian cells at physiological concentrations
(ii) biodegradable as it can be degraded by lysozyme and bacterial enzymes
(iii) mucoadhesive as it possesses amino and hydroxyl group structures that can interact with mucin through hydrogen bonding
(iv) Intrinsically immunomodulatory because it activates innate immune responses through pattern recognition receptors [37]. The properties are suitable for the use of chitosan in the drug delivery system, especially for targeting intracellular pathogens.

Table 2: Key Characteristics of Chitosan Relevant to Drug Delivery Systems

No.	Characteristic of Chitosan	Description / Significance
(i)	Biocompatibility	Chitosan is non-toxic to mammalian cells at physiological concentrations.
(ii)	Biodegradability	It can be degraded by lysozyme and bacterial enzymes.
(iii)	Mucoadhesivity	The presence of amino and hydroxyl groups enables interaction with mucin through hydrogen bonding.
(iv)	Intrinsic immunomodulatory property	It activates innate immune responses through pattern recognition receptors.

4.2 Synthesis of Chitosan Nanoparticles

Several methods have been proposed for the synthesis of chitosan nanoparticles, which have their own advantages and disadvantages [38].

4.2.1 Ionic Gelation

The most popular method for the synthesis of chitosan nanoparticles is the ionic gelation, or polyelectrolyte complex method [39]. The electrostatic attraction force between the positively charged amino groups of chitosan and the negatively charged groups of counter-polyanions, such as sodium tripolyphosphate (TPP), sodium alginate and DNA, are the basis of this method [40]. The process is simple, environmental friendly (no organic solvents used) and nanoparticles with high EE and stability are produced [41]. The chitosan/TPP ratio, pH, ionic strength and mixing rate can be used to adjust the particle size of the formulation [42].

4.2.2 Coacervation

Coacervation is a controlled pH complexation of chitosan with anionic polymers, e.g., sodium alginate or

carboxymethyl cellulose [43]. This technique is capable of controlling the particle size distribution and surface characteristics. This is because the resulting nanoparticles are more stable and uniform than the ionic gelation products [44].

4.2.3 Emulsification-Solvent Evaporation

This approach consists of dissolving chitosan in an organic solvent in this case it is acetic acid in water), emulsifying this solution in a non-aqueous solvent, then evaporating the organic solvent [45]. This method allows for high drug encapsulation and controlled nanoparticles size and morphology [46]. But it's possible that there's some leftover organic solvent that could affect biocompatibility.

4.2.4 Other Synthesis Methods

Reverse micelle approach, spray drying, and electrospray generation are alternative methods that are used. Each method has its own distinct advantages depending on the application and has the ability to tune the properties of the Nanoparticles [47].

5. FUNCTIONALIZATION OF CHITOSAN NANOPARTICLES FOR ACTIVE TARGETING

The ability to target molecules to be bound to the chitosan nanoparticles allow for active receptor-mediated delivery of the material to a specific cell type or disease site [48]. This strategy greatly improves therapeutic index by delivering therapeutic agents to their target sites eliminating non-target exposure to tissues and organs [49].

5.1 Antibody-Mediated Targeting

Cell surface antigen antibodies to macrophages or TB infected cells have been attached to chitosan nanoparticles [50]. Antibodies against the mannose receptor and against CD64 have been used as have antibodies against scavenger receptors [51]. These antibody-functionalized nanoparticles are able to penetrate cells via antibody-mediated receptor binding and cross-linking, possibly through antibody-dependent cellular phagocytosis [52].

5.2 Peptide and Protein Targeting

To improve the cellular penetration, the chitosan nanoparticles have been conjugated with cell-penetrating peptides (CPPs), such as TAT peptide, arginine-rich peptides (R8 peptide), etc. [53]. These CPPs are involved in the transport across biological membranes by energy independent transport and endocytosis [54]. Another class of mannose-binding peptides designed to bind to mannose receptors on macrophages makes use of the mannose-receptor's natural pattern-recognition capacity [55].

5.3 Carbohydrate-Based Targeting

Macrophages are equipped with pattern recognition receptors such as scavenger receptors, TLRs and mannose receptors that are able to efficiently bind to carbohydrate ligands [56]. The mannose and glucose coated chitosan nanoparticles and the chitosan nanoparticles coated with lactoferrin target moieties are taken up more by macrophages [57]. This is in line with higher uptake that has been reported for mannose-functionalized nanoparticles (2-5 fold higher uptake than non-targeted formulations) [58]. The strategy makes use of this natural immune surveillance property of macrophages to achieve therapeutic result.

5.4 Chemical Conjugation Methods

Various chemical strategies have been used to target ligands to chitosan nanoparticles: (i) carbodiimide coupling (EDC/NHS) for peptide/protein coupling; (ii) thioamide bond formation (2,2'-dithiodipyridine); (iii) click chemistry and azide-alkyne cycloaddition; (iv) maleimide coupling; and (v) disulfide bond formation [59]. The choice of a conjugation method depends on the type of a ligand to be used, the strength of such a bond (permanent versus cleavable) and compatibility with the drug payload [60].

6. MECHANISMS OF CELLULAR UPTAKE AND CELLS RELEASE OF DRUGS

The mechanisms of cellular uptake are important to consider when designing nanoparticles and to predict their in vivo efficacy [61].

6.1 Endocytosis Pathways

Chitosan nanoparticles are taken up by the cells through endocytic pathways, such as clathrin-mediated endocytosis, caveolin-mediated endocytosis and macropinocytosis [62]. The positive charge of chitosan allows non-specific interactions with the negative charged cell surface, thus promoting adsorptive endocytosis [63]. Mannose receptors are involved in the uptake of mannose functionalized nanoparticles, which are identified by mannose containing ligands [64].

The endocytic pathway starts with the formation of early endosomes that then mature into late endosomes that fuse with lysosomes to make an acidic phagolysosome [65]. The acidic microenvironment of lysosomes (pH 4.5-5.5) can cause degradation of nanoparticles and release of the drug in several ways:

(i) protonation of the amino groups of chitosan
(ii) enzymatic degradation of chitosan by the lysosomal enzymes
(iii) hydrolysis of pH-sensitive linkers or conjugates [66].

6.2 Escape from Endosomal Compartments

A key issue in the delivery of drugs into cells is the ability to escape endosomes, since many drugs are trapped in acidic endosomes and lysosomes, which are inaccessible to cytoplasmic and/or nuclear targets [67]. Chitosan has ‘proton-sponge' properties as it is able to bind protons released on acidification of endosomal compartments, leading to endosomal disruption [68]. This property allows PZA to escape from endosomal compartments into the cytoplasm where it will act on intracellular mycobacteria [69].

Other approaches to improve endosomal escape are the co-delivery of fusogenic lipids, the co-encapsulation of lytic

peptides and the use of pH-responsive polymers that change their conformation upon acidic pH [70]. All of these methods are useful for increasing the cytoplasmic bioavailability and therapeutic activity of these compounds [71].

6.3 Intracellular Localization and Drug Release

After being internalized, nanoparticles are sorted into diverse intracellular compartments, depending on their surface chemistry, size and cell environment [72]. In contrast, the cationic nanoparticles are more strongly bound to organelles that carry a negative charge such as the mitochondria and lysosomes [73]. In the case of TB, it might be beneficial to have localization within phagolysosomes as (i) M. tuberculosis replicates within phagosomes; (ii) the acidic environment of the phagolysosomes is required for the action of PZA; and (iii) concentrations in the phagolysosomes may be significantly higher than in the cytoplasm [74].

There are several mechanisms by which the release of the drugs from the nanoparticles can be triggered:

(i) diffusion-controlled release which depends on the solubility of the drugs and porosity of the nanoparticles
(ii) enzymatic degradation of the polymer matrix
(iii) pH-triggered release in the acidic compartments
(iv) triggered release in the presence of reactive oxygen species (ROS) or reducing agents [75]. The release of drugs can be temporally controlled, achieving adequate levels in the cells and reducing off-target effects.

7. PRECLINICAL EVIDENCE: IN VITRO & IN VIVO STUDIES

7.1 In Vitro Efficacy Studies

The effectiveness of PZA loaded chitosan nanoparticles has been shown to be greater than that of PZA alone in several in vitro models of macrophage infection [76]. In both cell culture systems, with luminescent or GFP-expressing M. tuberculosis strains, formulations of nanoparticles resulted in 2-8 fold higher intracellular drug levels relative to free drug in murine macrophages (RAW 264.7) and in human monocyte-derived macrophages (MDMs) [77]. This led to more effective reduction of intracellular mycobacterial burden with these formulations compared to free PZA at the same dose [78].

The ability of PZA loaded nanoparticles to decrease the survival and persistence of M. tuberculosis inside macrophages, specifically in phagolysosomes, has also been demonstrated [79]. In addition, combination products that combine PZA with isoniazid (INH) or rifampicin (RIF) exhibit synergistic antimycobacterial properties [80]. Interestingly, there are some reports stating that nanoparticle based delivery shows a lower toxicity in macrophages than free drug, indicating that the nanoparticles might have a better safety profile [81].

7.2 In Vivo Efficacy in TB Models

The PZA-loaded chitosan nanoparticles have been evaluated using various animal models, including mice infected with aerosolised M. tuberculosis [82]. Aerosol infection models have a high level of relevance to human TB transmission and disease, and therefore are very useful for pre-clinical evaluation [83]. In M. tuberculosis infected C57BL/6 mice, M. tuberculosis H37Rv and other strains have been shown to give better reduction in bacterial burden in the lungs with equivalent doses of nanoparticles than with the free drug [84]. Moreover, these formulations have been proved to make combination anti-TB regimens more effective [85].

The following are some key advantages that were seen in vivo: (i) long-lasting therapeutic activity that results in lower dosing frequency; (ii) better clearance of the bacteria from the lungs and spleen; (iii) less toxicity of the drug in the liver and kidneys than free PZA; and (iv) penetration of the granulomatous lesions was enhanced[86]. Notably, some studies have shown that, when TB is treated with a nanoparticle formulation, it has the potential to shorten the course of treatment needed for a cure, and thus, improve patient adherence [87].

8. SYNERGISTIC COMBINATION THERAPIES

PZA is a part of the standard TB treatment, but can also be combined with other novel approaches by being incorporated into nanoparticulate formulations [88]. Multiple anti-TB drugs can be co-delivered in a single nanoparticle, with several advantages linked to the concept:

(i) simultaneous delivery to target sites
(ii) optimum ratio of drugs for synergistic activity
(iii) prevention of "differential kinetics,"
(iv) potential shortening of treatment duration [89].

8.1 PZA-INH Combinations

The other first-line anti-TB drugs that have bactericidal activity against both rapidly growing and non-replicating mycobacteria are isoniazid (INH) [90]. Like PZA, INH is a prodrug that must be activated by catalase-peroxidase (KatG) [91]. Synergistic antimycobacterial activity has been demonstrated for PZA-INH dual loaded nanoparticles both in vitro and in vivo in murine TB models [92]. The mixture is especially effective against non-replicating mycobacteria, which are a major problem in the treatment of tuberculosis [93].

8.2 PZA-RIF Combinations

Rifampicin (RIF), the most potent anti-TB first-line drug, has a rapid bactericidal activity [94]. PZA-RIF loaded nanoparticles have been developed and these nanoparticles have been demonstrated to have enhanced antimycobacterial activity from free drug combinations [95]. One significant advantage of this combination is that the hydrophobic property of RIF facilitates the incorporation of PZA into the chitosan nanoparticles, which is likely to improve its encapsulation as well [96].

8.3 Triple and Quadruple Drug Combinations

Recent works have investigated encapsulations of 3-4 anti-TB drugs in a single nanoparticle [97]. Synthetic formulations containing PZA, INH, RIF and ethambutol (EMB) have been created by a modified ionic gelation method [98]. The multi-drug nanoparticles have the potential to substantially reduce the TB treatment from 6 months to as short as 2-3 months in animal models [99]. These reductions may significantly increase the likelihood of cure for TB patients, particularly by increasing adherence [100].

9. HOW TO OVERCOME DRUG RESISTANCE BY USING NANOPARTICULATE DELIVERY

The problem of drug resistance in TB is very big, with an estimated 484,000 MDR-TB cases in the world in 2021. Nanoparticulate delivery systems have special strategies for overcoming resistance [101].

9.1 Overcoming Resistance through Enhanced Bioavailability

A mechanism of drug resistance is decreased cell accumulation of the drug resulting from change in drug transporters or metabolic inactivation [102]. Increasing drug efflux or drug metabolism can be compensated for by maintaining intracellular levels high with the use of nanoparticles, which avoid the normal transport systems [103]. For instance, receptor-mediated endocytosis of mannose-targeted nanoparticles is not dependent on drug transport capability, which may make such nanoparticles useful in circumventing transporter-mediated resistance [104].

9.2 Combination Strategies Against Resistant Strains

PZA synergistic effect can also be maintained to the TB resistant strains when PZA is used in combination with second-line agents inside nanoparticles [105]. Also, co-delivery of PZA with agents that target resistance mechanisms (e.g., RIF plus agents that target RIF-resistant mutations) may be useful [106].

9.3 Immunomodulation to Enhance Host Defence

The nanoparticles made of chitosan have immunomodulatory properties, which increase innate immune responses [107]. These nano particles induce an increase in the pro-inflammatory cytokines (TNF-α, IL-6, IL-12, IL-18) and Th1 cell differentiation, both of which play a vital role in control of mycobacteria [108]. This immunostimulatory effect is in addition to the killing mediated by drugs and could contribute to the reduction of the bacterial load both by drug and immune-mediated mechanism [109]. This immune boosting action could be useful to avoid or retard the development of resistance [110].

10. CONCLUSION

Despite the success of global efforts to eradicate TB, the disease is still a major public health problem in the world, with the number of drug-resistant strains rising, which requires innovative treatment strategies. Although pyrazinamide is active against TB and it is a first-line drug, it has bioavailability and toxicity limitations that limit its clinical usefulness. Chitosan based nanoparticles provide a promising platform technology to overcome these limitations because of their ability to enhance intracellular delivery of drugs, immunomodulatory effects and rational multi-drug combination therapy. In both models of macrophage infection and TB murine models, extensive evidence has shown that chitosan nanoparticles containing PZA are more effective than free PZA. The functionalization with targeting ligands allows for targeted delivery to TB infected macrophages and further improves therapeutic index. The recent developments in combination therapy, immunomodulation, and overcoming drug resistance make chitosan nanoparticles a promising tool in the anti-TB arsenal.

But there are still many hurdles that need to be overcome before chitosan nanoparticles can be applied in the clinic.

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