

Review

New chimeric anti-tubercular dendrimers with self-delivering property

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Tuberculosis is the second lethal infectious disease caused by *Mycobacterium* species. This pathogens could cause severe disease like tuberculosis and leprosy in human. Today there are a number of anti tuberculosis agents utilized in treatment of this disease but multiple drug resistance is one of the major problems that end to failure in treatment. Dendrimers are synthetic, high branched polymers with a number of functional groups that could bind to different macromolecules like drugs, oligosaccharides that makes it appropriate for target drug delivery. Today, some type of dendrimers like Jeffamines are developed possessing self antimicrobial activity. Hypothesis: we propose that combination of ethambutol with 3rd generation of Jeffamine core based dendrimer (P3) and mannose could both create a complex compound with high potency against *mycobacterium* species that is targeted to macrophages via interaction between lectin receptors on immune cells and mannose molecules on outer branch of P3.

Key words: Tuberculosis, dendrimers, Jeffamines, *Mycobacterium*, ethambutol

INTRODUCTION

Tuberculosis (TB) is the second most lethal infectious disease (Sosnik et al., 2010). TB is an infectious disease that could be fatal caused by mycobacteria, generally *Mycobacterium tuberculosis* in humans (Vinay et al., 2007). The most contaminated part of body is attributed to lungs even though other parts are also infected to some less extent. Unfortunately the most con-taminations remain undiagnosed because the infection is in latent phase and only 10% of them become active that could take the life of patients in 50% of cases if untreated. The usual ways of spreading is through coughing, sneezing or spit (Konstantinos, 2010). The most common symptoms comprising fever, weight loss, and chronic cough associated with blood tinged sputum and night sweats. The rate of infection in the world is increasing that is typically in developed countries (WHO,

2009).

Concepts

We propose that ethambutol (an antimycobacterium agent) could be replaced with one or several of the functional groups on the branches of P3, which also possess antimycobacterium effect parallel to self-target delivery property meanwhile a mannose is bound to one or several other branches (Figure 1) that could interact with its receptors on macrophages resulting in development of a highly potent compound that is targeted to immune cells properly.

Mycobacterium tuberculosis

Mycobacterium is a member of Actinobacteria class. This group of pathogens accounts as hazardous bacteria and could potentially cause severe diseases like tuberculosis and leprosy in mammals (Ryan and Ray, 2004). As introducing in body, they could present no significant signs. For this reason, today many people

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Abbreviations: TB, Tuberculosis; PAMAM, polyamidoamine; CD, cyclic oligosaccharides; CFUs, colony-forming unit

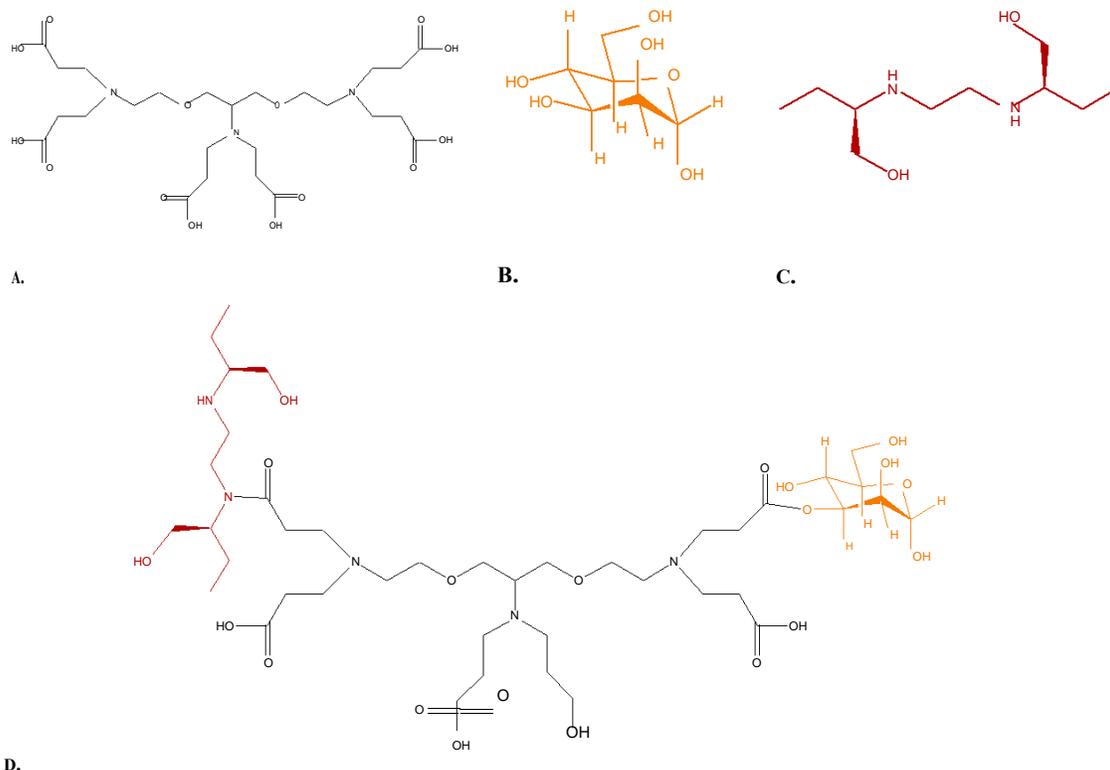


Figure 1a. The structure of 3rd generation of Jeffamine core based dendritic (P3), b. The structure of mannose, c. The structure of ethambutol, d. The general structure of 3rd generation of Jeffamine core based dendritic (P3), which also possess antimycobacterial effect parallel to self target delivery property, in combination with ethambutol and mannose as novel antimycobacterial agent.

around the world are infected with *M. tuberculosis* while they know nothing about their disease. *M. tuberculosis* possesses a cell wall dissimilar to Gram negative and positive that is resistant to many antibiotics resulting in difficulty in treatment plane. In addition to cell wall that gives the pathogen unique properties against antibiotic drugs, other factors also are effective in virulence of this pathogen including its surface and secreted proteins (Woolridge, 2009). Invasion and replication into alveolar macrophages is the primary and main stages of disease that happens before this pathogen is distributed (Shirzad et al., 2008).

Drugs used against *Mycobacterium tuberculosis*

There are several problems in treatment of TB that essentially get backs to the unique structure of the TB that could cause drug resistance (Acharya et al., 1967; Migliore et al., 1966; Acharya and Goldman, 1966). Rifampicin and isoniazid are among the most frequently used drugs in the treatment of tuberculosis that should be administered for about 6 to 24 months. If a patient is diagnosed in latent infection, only one antibiotic is used but in active form, multiple drugs are exploited in order to lessen the rate of antibiotic resistance (CDC, 2003).

Ethambutol is another drug used as a bacteriostatic antimycobacterial drug prescribed in treatment of tuberculosis that is more explained in the next parts. One of the impediments in treatment of tuberculosis efficiently is the long time – period of treatment and high price of pills that could contribute to failure and development of multi drug resistant strains (Sosnik et al., 2010). Thus, strategies like design and development of novel formulations along with novel antimicrobial compounds that could reduce the length of treatment and reduce the resistance are inevitable.

Dendrimers

Dendrimers are synthetic, highly branched macromolecules with nanometer dimensions and are characterized by structural perfection (Anil et al., 2002). Advent of dendrimers as drug delivery systems in nanoscale has attracted much interest for studying controlled drug delivery systems (Umesh et al., 2006). They are monodisperse and usually highly symmetric, spherical compounds. The main features of dendrimers is related to functional groups on the molecular surface, though, some dendrimers possess internal functionality (Antoni et al., 2009; McElhanon and McGrath, 2000; Liang and

Fréchet, 2005). Dendrimers are investigated as carrier for drug delivery system (Anil et al., 2002). Among such diverse dendrimers, polyamidoamine (PAMAM) are much more studied. Researchers throughout the world investigated such dendrimers in different aspect. In one study (Malik et al., 2000) the relation between structure and biocompatibility of PAMAM has been studied. They reported that cationic dendrimers are not suitable candidate as drug carrier because of their hemolytic and cytotoxic properties at even relatively low concentrations as well as having short half life and are cleared quickly from the body. Contrary, anionic PAMAM dendrimers possess longer half life while every less generation number, the more half life. In one study, it was defined that with increase in molecular weight and size of dendrimers, the extravasation across microvascular endothelium is also enhanced. Because of the toxicity of cationic PAMAM it is essential to replace the amino groups on surface area with neutral or anionic groups to eliminate toxicity and prevent its accumulation in liver (Jevprasesphant et al., 2003). In another study (Bhadra et al., 2003), polyethylene glycol was added to dendrimer structure to improve drug delivery of anticancer agnates. By this way, it was found that PEGylated dendrimers dominated by some more advantages in comparison to previous generations. The first superiority is related to finding more drug loading capacity via creating more functional groups on the outer surface that lead to much more drug interaction. Second advantage is attributed to diminishing hemolytic toxicity of dendrimers. It has been demonstrated that dendrimers can pass through GI membranes and are also valuable while deliver in the GI is dependent to pH and enzyme. Dendrimer also could improve the pharmacokinetics profile of drugs. The positive role of dendrimers in transdermal flux and enhancement of corneal retention time is also proved. However, regardless of all these benefits, the toxicological features of dendrimers should not be ignored and must be evaluated comprehensively (Aulenta et al., 2003; Bosman et al., 1999; Esfand and Tomalia, 2001; Patri et al., 2002; Cloninger, 2002; Gillies and Frechet, 2005). In recent times, water-soluble dendrimers have been exploited for the targeting of rifampicin, chloroquine and lamivudine to macrophages for treatment of tuberculosis (Briones et al., 2008). Generally, small molecules are uptaken by different mechanism comprising phagocytosis, fluid phase pinocytosis or by receptor-mediated endocytosis. Since phagocytosis mechanism take places for molecules with at least 500 nm in size, cellular uptake of dendrimers are not dependent to phagocytosis as their size of 20-30 nm (Rupper and Cardelli, 2001). In addition, receptor-mediated endocytosis is ruled out when there is no ligand on the outer surface of dendrimer. Existence of -OH groups in outer surface of dendrimers disturb any ionic interaction with cell membrane (Chirila et al., 2002).

The only supposed mechanism for cellular uptake of

dendrimers is fluid phase endocytosis through non-specific interactions. This mechanism was verified when no dendrimer went through cells as fluid phase endocytosis inhibitor was added to the media (Kolhe et al., 2004). Eventually, after entering into cell, the polymer-drug conjugate would be cleaved by hydrolytic enzymes in the lysosomal compartment, producing high concentration of drug in cell (Llyod, 2000).

Lectin and oligosaccharides

Dendrimers mostly PAMAMs are considered as suitable candidates for delivery of anti-TB agents because of their unique structure. In order to target the drug delivery to macrophages Kumar et al. (2006) developed a novel generation of rifampin loaded dendrimers that were modified with mannose molecules on surface. Mannose molecules could bind to their receptors on macrophages that result in improved their uptake (Kumar et al., 2006). High haemolysis levels derived from amine-termina dendrimers could hinder their clinical application while mannosylation could dramatically decrease the hemolytic toxicity (Sosnik et al. 2010). In another study, combination of dendrimers with cyclic oligosaccharides (CD) was investigated. CD is composed of 12 D-(+)-glucopyranose units linked by α -(1-4) bonds. The CD has an outer hydrophilic and an inner hydrophobic part that permits hydrophobic molecules be placed in inner part resulting in elevated solubility (Loftsson and Duchene, 2007; Brewster and Loftsson, 2007). In one study, addition of CD to the dendrimers increased the solubility of the poorly-water soluble nitroimidazole P-824 as a new anti-TB drug (Lenaerts et al., 2005). Even though enhanced solubility was not observed with rifampin but such complexes could improve the thermal stability of the drug as well as enhanced passing of the drug through the wall of the bacilli (Lindenberg et al., 2004). Norfloxacin in solution is not effective against *M. bovis*, while its combination with a dextran polymer bound to mannose could lessen the number of colony-forming unit (CFUs) in the lungs of mice infected with *M. bovis* (Roseeuw et al., 2003).

Antimicrobial activity of water soluble dendritic macromolecules

Several types of water soluble dendrimers are designed and developed based of poly(propyleneoxide) amines (Jeffamines) (P1). P1-core and branched units were developed from both methylacrylate and ethylenediamine (P2-P9, and generations 0-3 with -NH₂, -COOH functionalities) (Metin, 2009). The unique architecture of dendrimers along with multiple functional groups gives impetus researchers to use such structures for designing as both antimicrobial agents (Chen and Cooper, 2000).

The above specific dendrimer is a dendritic poly-chelators starting from P1 as an initial core composed of amide connectivity without internal hydrolytic cleavage (Tomalia et al., 1986; Beezer et al., 2003; Newkome et al., 1992). The antimicrobial activity of Jeffamines is proved against some Gram-negative and Gram-positive such as *Mycobacterium smegmatis* (Wikler et al., 1993; Collins et al., 1989).

Ethambutol

Ethambutol is one of drugs utilized in treatment of TB bacilli. Its mechanism of action is through blocking the cell wall via inhibiting enzyme arabinosyl transferase that results in arabinogalactan synthesis disruption. Therefore, the permeability of cell wall would be increased (Yendapally, 2008). It is usually prescribes in combination with other anti-tubercular drugs, like isoniazid, and rifampicin.

DISCUSSION

Since TB is one of the life-threatening diseases and is spreading out in developing countries (Sosnik et al., 2010; Vinay et al., 2007) it is necessary to combine old drugs with new ones to produce high potent compounds to overcome multiple drug resistance that accounts for as one of the most important impediments in treatment of tuberculosis. Even though the role of dendrimers as drug delivery system have been reported by many researchers (Anil et al., 2002; Umesh et al., 2006; Antoni et al., 2009; Malik et al., 2000), but there are some type of dendrimers (Jeffamine core) with anti-tubercular properties (Metin, 2009) that could potentially be utilized in treatment of tuberculosis in a highly efficient manner. Therefore, it is concluded that combination of dendrimers and an anti-tubercular agent like ethambutol (Figure 1) could serve synergistic effect on tuberculosis species; even though targeting the dendrimer-ethambutol conjugate to desired immune cells like macrophages could also provide better effectiveness with improved therapeutic profile. To achieve this aim, exploiting mannose molecules as oligo-saccharide ligands on outer surface of dendrimers could target this complex to lectin receptors on macrophages. We think that using this strategy to practice, could facilitate tuberculosis treatment and prevent multiple drug resistance.

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