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Review Article

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Cholesterol Metabolism: As a Promising Target Candidate for Tuberculosis Treatment by Nanomedicine

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Abstract

We are facing a tremendous need to develop anti-tuberculosis (TB) drugs due to extreme rise in incidence and mortal cases of this disease. *Mycobacterium tuberculosis* (*M. tuberculosis*), the causative agent behind this malady have attained the drug-resistant characteristic by adding mutation at its genetic level and modifying their metabolic pathways. An important metabolic pathway employed in the bacterium is cholesterol metabolic pathway. Cholesterol is needed by the bacterium for attachment, entry, as a major nutrient source, persistence, and infection in the host. Manifold roles of cholesterol in *M. tuberculosis* making it an important mark to target the survival and virulence of the bacterium. Genetic regulation of cholesterol metabolism is a complex phenomenon. This review emphasizes the close and quick view towards cholesterol metabolism in *M. tuberculosis* and nanotechnology strategies to target this pathway. Targeting this pathway with specific biomarker designed nanoparticles loaded with anti-cholesterol drugs (Azasteroid, steroid, econazole, etc.) might be a better way of treatment. Antituberculosis drugs that could target their specific enzymes could lead to hindrance in uptake and degradation of this lipid and thus lead to nutrient depletion and accumulation of toxic metabolites which may ultimately lead to bacterial death.

Keywords

M. tuberculosis; Cholesterol; Mannose; Nano particle; Econazole; Dosage

Abbreviations

M. tuberculosis: Micobacterium Tuberculosis; TB: Tuberculosis; MDR-TB: Multiple Drug Resistant Tuberculosis; PLGA: Poly Lactic-co-Glycolic Acid; MFS: Major Facilitator Super Family; 3β-HSD: 3β-Hydroxysteroid Dehydrogenase; ChoD: Cholesterol oxidase; SDN: Solid Drug Nanoparticle; PLA: Poly-Lactic Acid; PCL: Poly-ε-Caprolactone; INH: Isoniazid; RIF: Rifampicin; PZA: Pyrazinamide; ETM: Ethambutol; cAMP: cyclic Adenosine Mono Phosphate; TACO: Tryptophan Aspartate Containinsg Coat Protein; LAM: Lipoarabinomannan; TDM: Trehlose-6,6'-dimycolate; PDIM: Phthiocerol Dimycocerosate; PGL: Phenolic Glycolipid; TAT: Twin Arginine Transporter System; SDS: Sodium Dodecyl Sulfate; ESX: Excretory System

Introduction

Mycobacterium tuberculosis H_{37}Rv (*M. tuberculosis*) which causes disease Tuberculosis (TB) in humans is one of the major reasons for death due to an infectious disease. In 2017, 10 million people were diseased with TB, and 1.6 million died from the disease. It is now known that *M. tuberculosis* survival mechanism depends upon many factors such as the layer of mycolic acid, dormancy and persistence, protection against oxidative radicals, Vitamin D3, cholesterol, and retinoic acid, etc. Vitamin D3 and cholesterol play crucial role in growth of the bacterium [1]. TB is currently decreasing with rate of 2% worldwide but the rate is needed to be increased up to 4%-5% to end this disease in the coming years. Major countries contributing to the widespread disease are developing countries or southern Asian such as India, Pakistan, China, Philippines, Indonesia, Bangladesh, South Africa, and Nigeria. TB mostly affects adults in their productive years but the disease can be found in every age group in developing countries, approximately 1 million children are living with TB and 230,000 deceased in year 2017 itself. The global prevalence of this disease is comprised of the current epidemic of co-infection of HIV and diabetes with TB. The condition is becoming much worse with an alarming increase in drug-resistant *M. tuberculosis* strains. Currently BCG vaccine is the only vaccine being used to cure the disease but is now becoming invalidated due to genetic drifts in nature of the bacterium. Despite availability of BCG vaccines, Isoniazid and rifampicin are other two primary first-line anti-TB first-line drugs that are widely used in treatment of this disease. *M. tuberculosis* employed an efficient phenomenon to rescue from host environment and additive mutation effect in their genome. These characteristics of this bacterium make treatment of the disease complex and give rise to generation of Multiple Drug-Resistant Tuberculosis (MDR-TB), XDR-TB and TDR-TB. Second and third-line drug regimens as shown in Table 1 are the extensive therapy to cure this disease by applying more antibacterial effect [2-15]. Although TB is caused by spreading of the bacteria through air channels while coughing and sneezing etc. But, it is seen that smoking is also attributing a significant percentage of 7.9% to the deadly disease. Along with it TB with HIV is common and confounded now and forms a deadly combination. People infected with HIV are 20 to 30 percent prone to TB rather than people without HIV [16]. In year 1882 the deadly disease was discovered and till now the disease is making its way to proliferate and even spreading on a large scale. Even after whole-genome sequencing been completed for this bacterium and more than 140 passed years, we are still unable to completely eliminate this disease. The notorious bacterium has very complex and rigid structure and employs several mechanisms to survive inside host cell. On the primary basis, it forms and resides inside a granulomatous lesion in the alveolar sacs for a longer period of time without any interference from host immune system. Granuloma is a cluster of immune cells that encircle or trap any foreign strain, which is not phagocytosed by macrophage cells. The immune cells that participate in formation of this granuloma are mainly highly differentiated cells such as epithelioid cells, multinucleated giant cells, B-cells, T-cells, macrophages cells that give granuloma a foamy appearance. Granuloma accomplishes a mutual relationship between host and bacterium, *M. tuberculosis* can stay inside the granuloma for years in dormant state and in time of immune-compromised state it can escape from the granuloma to proliferate which results into the symptoms of active disease. Despite residing inside the granulomatous lesion of the host cells, this bacterium applies several

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other mechanisms also to escape from host stress environment and defense mechanism (Figure 1). These responses include up-regulation of genes involved in stress response conditions, mutation in genome, modifying its metabolic pathway, etc [17].

The main focus which we try to elaborate and the major aim of this article is to exaggerate cholesterol metabolism in *M. tuberculosis* and how much it is important for its survival? If it is necessary to a great extent, then how can we target this metabolic pathway so that growth of this bacterium could be halted which ultimately leads to lesser epidemic of this disease? In order to focus on the mentioned research arm, we are aiming towards developing various strategies employed in the development of novel drug targeting catabolism of cholesterol of *M. tuberculosis* [18]. This would be done with the help of nanobiotechnology where nanoparticle would be loaded with various types of anti-cholesterol drugs. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles are best-known class of nanoparticles which are biodegradable and biocompatible polymers that are hydrolytically degraded into nontoxic monomers, oligomers, lactic acid, and glycolic acid. Nanoparticle would be loaded with first-line drugs such as isoniazid, rifampicin, pyrazinamide, ethambutol, econazole, and steroid fibrates. The drugs and steroids might play very important role in cholesterol metabolism and reducing the disease [19].

As mentioned in various reports and also in the above lines of this article, Cholesterol metabolism is one of the main and major arms that work for bacterial survival, growth, and virulence. Targeting this arm with the help of nanoparticle loaded with effective drug turn out to be an efficient technology towards eradicating or minimizing the threat of this disease.

The fundamental role of cholesterol in *M. tuberculosis*

Mycobacterium resides inside alveolar macrophages in a very nutritionally constrained environment and has capability to persist in this environment for many years. It had been said that most of the bacteria utilize the catabolite repression phenomenon in which they prefer to use one substrate for growth over the other [20]. But as most of the bacterial species, *Mycobacterium* does not obey the same rule of catabolite repression. This bacterium evolved with the ability to co-catabolite different carbon sources including acetate, dextrose, glycerol, etc. with different metabolic fate which ultimately leads to maximization of its monophasic growth [21].

There are various reports which proved that intracellular pathogens obtain their nutrient from surrounded lipids of the host and they utilize these lipids as their primary carbon source rather than using carbohydrates. Host cells comprise different types of lipids and one of the major lipids that present is cholesterol. Cholesterol accompanies a major part of lipid in eukaryote cell and thus this is also a major lipid from which *M. tuberculosis* obtains its nutrients [22]. This was also proved by a study that shows impair growth of *M. tuberculosis* in the absence of cholesterol. Cholesterol is majorly required for persistence of the bacterium in addition to establishing and propagating infection inside host cells [23]. The impaired growth of the bacterium in the absence of cholesterol is due to non-functioning of *mce4* operon. *mce4* operon consists of various genes that form a part of ABC transporter family and are majorly responsible for cholesterol uptake. This statement was confirmed by the study of Sanaratne et al. which showed that mce4 mutants were unable to grow on medium where cholesterol is the sole carbon source. In addition, cholesterol is not just taken up by *M. tuberculosis* but also catabolized.

Ramon Garcia et al. confirmed the role of Rv1410c which codes for P55 efflux pump in *M. tuberculosis* growth in the presence of cholesterol. Another study also proved the interaction of mce4 and mce1 with P55 gene in *in-vivo* conditions. P55 gene is a part of membrane transporter of major facilitator superfamily (MFS) [24]. This gene mainly benefited the bacterium by maintaining cellular growth of *M. tuberculosis*, maintaining its intracellular redox balance, etc. This gene was found to be a major culprit with regard to exportation of antibiotics, primarily first-line antibiotics against TB which leads to development of drug-resistant TB. P55 gene is also an important part of P55-lprG operon in which LprG gene encoded by Rv1411c. Rv1410c-lprG deletion mutant had shown attenuated characteristics of *M. tuberculosis* growth in mice [25].

Despite the advantages of cholesterol, it is required for retention

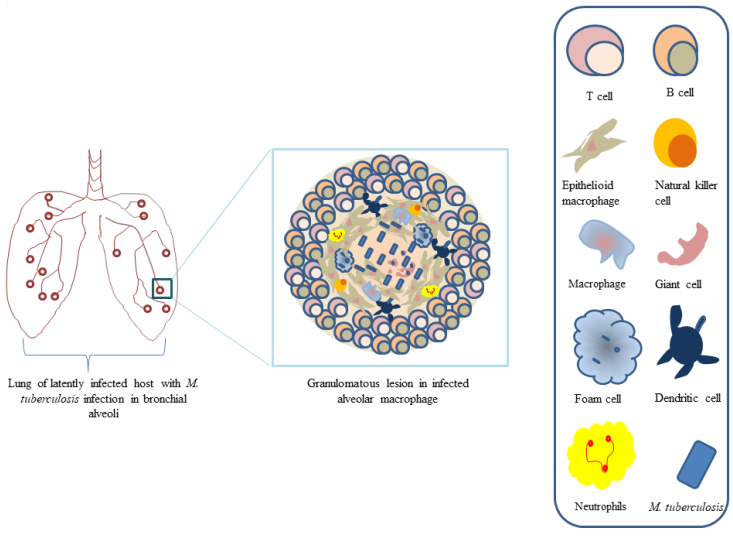

Figure 1: Establishment of granuloma: Infection of *M. tuberculosis* begins with inhalation of bacilli during contact with an infectious person. Bacterium resides into alveolar macrophage where it establishes a mutual relationship with host in form of granuloma. In granuloma, *M. tuberculosis* can reside in persistent manner without interference of host and on the other side host immune cells (B cell, T cell, NK cell, macrophage, dendritic cell, neutrophils etc.) invade bacterium and defend host from its diffusion.

of TACO on *M. tuberculosis* containing phagosome and due to this retention, only the fusion of phagosome lysosome is blocked [1]. As lipid plays major role in *M. tuberculosis* infection in different ways thus targeting cholesterol uptake of the bacterium provides us with much greater extent of the treatment. Hence, the above-mentioned information indeed shows that cholesterol is one of the factors which affect the growth of *M. tuberculosis* and very important role in persistence and virulence of this bacterium.

Genetic regulation of *M. tuberculosis* cholesterol metabolism

Although it had been said from many decades that cholesterol is essential for mycobacterial survival, infection as well as for persistence, the acquisition, and its utilization mechanism remains poorly described. The majority of studies covers *M. tuberculosis* and cholesterol as their main topics evidence about the utilization of host cholesterol by bacterium as a source of carbon and energy. First of all uptake of cholesterol require mce4 operon which spans from Rv3492c to Rv3501c in the mycobacterial genome [26]. The bioinformatic analysis employed in several previous studies proved that there are 51 genes out of 82 genes cluster are consistently expressed during growth on cholesterol. One of them is kstR (Rv3574) encodes a tetR like transcriptional repressor and regulate cholesterol metabolism, another gene in the operon is kstR2 (Rv3557c) which is responsible for differential expression of 15 genes (Rv3548-Rv3565) [27]. One of the famous regulatory processes for cholesterol metabolism is beta-oxidation and the main enzyme responsible for this is fadA5 which catalyzes thiolysis of acetoacetyl CoA and activity of this enzyme leads to generation of androsterone metabolites required for virulence [28]. One of the recent studies found the essentiality of fad operon (fadD17, fadD19, fadE26, fadE27, and rv04690) in *R. rhodococcus* cholesterol side-chain degradation [29]. There are other genes also that take part in sterol ring degradation in cholesterol regulation such as Rv1106c and Rv3409c code for a 3β-hydroxysteroid dehydrogenase (3β-HSD) and cholesterol oxidase (ChoD) respectively. These enzymes help in cholesterol metabolism by degrading cholesterol to cholest-4-en-3-one. The other catabolic gene is hsaD is a part of operon that consists of hsaA, hsaC and hsaD which is required for survival of the bacterium inside macrophages is member of α/β Hydrolase family responsible for aromatic compounds degradation [30].

Apart from this genetic regulation, there is a cytochrome p450 system is present in *M. tuberculosis* that is responsible for major steps for β-oxidation. Saturation of side chains of cholesterol is the necessary step for β-oxidation. Multiple steps have been required to complete the side chain of cholesterol and these steps are as oxidation of cholesterol to alcohol, aldehyde, and acid and all these conversions requires action of cytochrome p450 [31]. Three genes of cytochrome p450 system (CYP125; Rv3545c, CYP142; Rv3518c and CYP124; Rv2266) have been required to complete this function as mentioned in Table 2.

Designing of nano bio-particle to target *M. tuberculosis* $H_{37}Rv$

The concept of using nanoparticle is adequate and much benefited as it makes a drug to target at the site of infection, with least or minimal side effects and a quicker way to act upon the bacteria. It acts as a self-driving vehicle to release or unload the drugs to specifically target site. Delivery of drugs with nanoparticles could be advantageous in form of giving temporal and target-dependent transportation of drugs. These nanoparticles comprises of relatively large surface area to volume ratio which makes it feasible to make contact with intracellular parts either through the surface or internally. Nanoparticles in the field of medicine could be used in form of nanocarriers, polymer therapeutics and Solid Drug Nanoparticle (SDN). Nanocarrier could be used as

Table 1: List of all drugs that had been used to cure Tuberculosis (TB).

	Drug	Mode of action	Invention	Reference
First Line Drugs	Isoniazid	Inhibit cell wall, nucleic acid synthesis, causes metabolic depression by activation through katG that generates NAD(+) and NADP(+)	1952	2
	Rifampicin	Inhibit bacterial RNA polymerase	1966	3
	Ethambutol	Inhibit synthesis of cell wall component arabinogalactan and Lipoarabinomannan (LAM) by inhibiting arabinosyltransferases: EmbA, EmbB, and EmbC	1961	4
	Pyrazinamide	Inhibit membrane transport and disrupt membrane potential	1952	5
Second Line Drugs	Ethionamide	Disrupts mycolic acid biosynthesis and responsible for inhibiting acid fastness	1956	6
	Prothionamide	Disrupts mycolic acid biosynthesis and responsible for inhibiting acid fastness	1956	6
	Capreomycin	Inhibit mycobacterial protein synthesis by interruption in binding with inhibit 30S and 50S ribosomal subunits	1960	6
	Bedaquiline	Inhibit ATP production by binding to mycobacterial ATP synthase	2012	7
	Kanamycin	Inhibit protein synthesis by binding with 30S subunit of ribosome	1957	6
	Levofloxacin	Inhibit DNA gyrase and DNA topoisomerase IV	1990	8
	Moxifloxacin	Inhibit DNA gyrase and DNA topoisomerase IV	1990	8
	Para-aminosolicylic acid	Inhibit the action of dihydrofolate reductase	1943	9
Third Line Drugs	Bedaquiline	Inhibit ATP production by binding to mycobacterial ATP synthase	2012	7
	Delamanid	Inhibit synthesis of methoxy mycolic acid and ketomycolic acid component of cell wall	2014	10
	Clofazimine	Competitive inhibitor of menaquinone, a cofactor of mycobacterial ETC and release reactive oxygen species	1986	9
New Drug, B Candidate	SQ-109	Inhibit activity of MmpL3		10
	Linezolid	Bind with 50s subunit and inhibt formation of initiation complex of protein synthesis	1980s	11
	Rifapentine	Inhibit mycobacterial DNA polymerase		12-13
	Meropenem	Inhibit cell wall synthesis		14
	Imidazopyridine Amide	Inhibitor of mycobacterial Electron Transport Chain		14
	Macrolide	Inhibit protein synthesis by binding to the 50s subunit		15

transporters for drug candidates that are either encapsulated inside or attached to surface by strong covalent interactions. The most famous nanocarriers are lipid nanoparticle that provides colloidal stability to the encapsulated hydrophilic drug. Polymer therapeutics is conjugate compound of polymer and drug which releases the drug upon interaction with the target site. This type of nanoparticle could provide physiological distribution as it could be made specified to specific cells. This specific transport could be possible by designing nanoparticle with specific type of biomarkers that only recognizes paratope present on antigen surface or it can also make pH-responsive element that only activates and release drug at low pH. Solid drug nanoparticle on the other side is the drug itself with modified surface adsorbed material. This is the simplest form of nanoparticles that comprises large surface area to volume ratio thus provides greater extent to expose drugs in comparison to non-formulated native drugs [32].

There is a different class of biodegradable nanoparticles that have been discovered till date such as Poly-d,l-lactide-co-glycolide (PLGA), poly-lactic acid (PLA), Poly-ε-caprolactone (PCL), Chitosan, gelatin, etc. The nanoparticle surface should be designed in such a way so that it functionalized only on the confluence of infected alveolar macrophage cells infected with *M. tuberculosis* and treat only infected cells without any harm to its neighboring parts. This could be possible by designing nanoparticles with D-mannose at optimum concentrations and put the whole mixture for agitation. This procedure thus helps in the targeted delivery of drugs only to the *M. tuberculosis* cell as bacterium express mannose receptors on their surface [1].

Loading of the nanoparticle with first-line drugs would be a better way to treat the infection at primary level. There are several medications had been developed to cure this malady such as isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), ethambutol (ETM), bedaquiline, capreomycin, delamanid, kanamycin etc. There are other drugs that are used to treat drug-resistant tuberculosis as well, Table 1 enlists all drugs that have used to treat TB at different stages [33-40].

Advancement to target cholesterol metabolism by nanoparticle

After completion of organization of the desired vehicle, it is loaded with the required arms and weapons to attack the pathogenic strain but still modifications and advancement will be required as we know *M. tuberculosis* is a notorious bacteria it mutates itself according to the stresses. We need to introduce the concept of dosage, time-dependent manner of drug delivery and path of administration are three aspects that are very much important. We know that drugs or steroids work best at some optimal level beyond that or below it, their efficiency decreases significantly. Time-dependent manner of drug delivery is very important and different from dosage because we intend to maintain/exhibit the constant stress created by previous doses of drugs and another important factor would be that how much time then would be taken by our nanoparticle to degrade. Temporal dependent regulation also helps other uninfected cells to get sudden and frequent harm from large amounts of drugs. After setting all above parameters, the nanoparticle loaded with drug might target *M. tuberculosis* cell and inhibit cholesterol uptake as well as its

Table 2: Genes involved in regulation of cholesterol metabolism in *M. tuberculosis*.

Functional category	Gene	Mycobacterial homolog	Molecular function
Cholesterol Import	mce4A (Rv3499)* mce4B(Rv3498)# mce4C (Rv3497)# mce4D (Rv3496)# mce4E (Rv3495)# mce4F (Rv3494)#	*M. bovis*#* *M. leprae** *M. smegmatis*#* *M. marinum*#*	Transport cholesterol across mycolic acid and pseudoperiplasmic space
	YrbE4 (Rv3501)* YrbE4B (Rv3502)*	*M. bovis** *M. leprae** *M. smegmatis** *M. marinum**	Transport cholesterol through cytoplasmic membrane
	Mam 4A(Rv3493)* Mam4B (Rv3492)*	*M. bovis** *M. smegmatis** *M. marinum**	Regulator of mce4 assembly
Degradation by β Oxidation	Cyp 125 (Rv3545)* Cyp142 (Rv3518)*	*M. bovis** *M. smegmatis** *M. marinum**	Hydroxylation of terminal C of cholesterol
	FadD19 (Rv3515)* FadA5 (Rv3546)#	*M. bovis*#* *M. leprae** *M. smegmatis*#* *M. marinum*#*	Add CoA to the side chain
	ChsE4(Rv3504)* ChsE3 (Rv3572)* ChsE1 (Rv3543)# ChsE2 (Rv3544)&	*M. bovis*#&* *M. leprae** *M. smegmatis*#&* *M. marinum*#*	component of multimeric acyl-CoA dehydrogenase complex
	Ltp2 (Rv3505)*	*M. bovis** *M. leprae** *M. smegmatis** *M. marinum**	Work as an aldolase enzyme and cleave C-C bond
Degradation of A and B ring	KstD (Rv3537)& KshA (Rv3526)# KshB (Rv3571)* hsaB (Rv3567)#	*M. bovis*#&* *M. leprae** *M. smegmatis&#** *M. marinum#**	Degrades side chains A and B ring
Degradation of C and D ring	FadD3 (Rv3561)$ IpdA (Rv3551)# IpdB (Rv3552)# FadA6 (Rv3556)#	*M. bovis$#* *M. smegmatis#* *M. marinum$#*	Degrades side chains C and D ring

*, #, & shows the relative presence of mycobacterial homolog's in respective *Mycobacterium* species

metabolism which ultimately leads to intoxication due to cholesterol metabolites (Figure 2).

Fibrates are the other alternatives that can be used because *M. tuberculosis* body structure contains a layer of lipids and fatty acids below mycolic acid where fibrates could provide vulnerable stress to the pathogenic bacteria and might degrade the primary level defense mechanism of *M. tuberculosis*. With the help of first-line defense drugs and fibrates, *M. tuberculosis* is exposed to sudden stresses and it thus reduces the cholesterol uptake by *M. tuberculosis* by the help of econazole which acts as the carbon source and helps in growth and proliferation of bacteria. The dosage of aimed medicine should fall short of the multiplication time of *M. tuberculosis* so that it doesn't give time to bacteria to multiply and develop any kind of resistance to the medicine. The path of administration or drug delivery should be nasal (which can be inhaled from mouth or nose) as we aim to target the alveolar sac where the macrophage cells hold up the pathogenic strain. In this section the selection of nanoparticle matters the structure and designing plays a very important role as we aim our drug to be inhaled through nasal cavity according to which chitosan, PLGA proves to be the best nanoparticles [41]. Cyclic adenosine monophosphate (cAMP) on the other side, also inhibits mycobacterial cholesterol metabolism by inhibiting initiation steps of its degradation pathway and therefore prohibits bacterial replication inside macrophages [42]. But the main focus is to target cholesterol uptake and its metabolism in the *M. tuberculosis* cell. Econazole, LP10 azasteroids, etc. are some of the dugs that act upon cholesterol metabolism by interfering with cytochrome P450, cyp125 and sterol biosynthesis respectively enlisted in Table 3 [43]. After selection of drugs to be loaded in the nanoparticle, the next step would be to regulate dosage and temporal modification which is possible by maintaining the thickness of outer layer of nanoparticle so that it releases small amount of drugs at regular interval of time and thus leads to less cytotoxicity and harm to the other cells [32].

What else could be considered on the multidrug-resistant strain of *M. tuberculosis*?

Although we have tried to limit our approach till inhibiting cholesterol uptake and metabolism but there are other factors also that add in the virulence of this bacterium. Those factors are lipid content of the cell wall, vitamin D3, retinoic acid, retention of Tryptophan aspartate containing coat protein (TACO) protein, dormancy, and persistence within host macrophage, the protection against oxidative radicals, etc.

Optimum translocation of Lipoarabinomannan (LAM) into the cell wall is an essential feature in comprising mycobacterial stability. In addition to increasing stability, LAM also participates in the arresting of phagosome maturation by immature rab5 marker that also marks intracellular survival of the bacterium. It also triggers response of cytokines upon infection. Lipomannan is another constituent lipid of mycobacterial cell wall that is responsible for innate immune response against pathogens also important for virulence.

Trehlose-6, 6'-dimycolate (TDM) is another important part of virulence that is known as cord factor is the most abundant and toxic lipid in the mycobacterial cell wall envelope. It is comprised of two trehalose head groups and esterified mycolic acid molecule. This lipidomic content variation is the major determinant of inflammatory response. In spite of being functional to generate inflammatory responses, this molecule also responsible for inhibiting acidification of phagolysosomes, inhibit calcium-dependent fusion of phagosome-lysosome that is the main feature for killing of pathogen within the initial phase of infection. Phthiocerol dimycocerosate (PDIM) and phenolic glycolipid (PGL) are lipids that are responsible for duplication of bacterial cells. PDIM is also involved in mycobacterial resistance to detergent and also protect bacterium against reactive nitrogen and oxygen species.

Although the lipid profile of *M. tuberculosis* is the main focus for

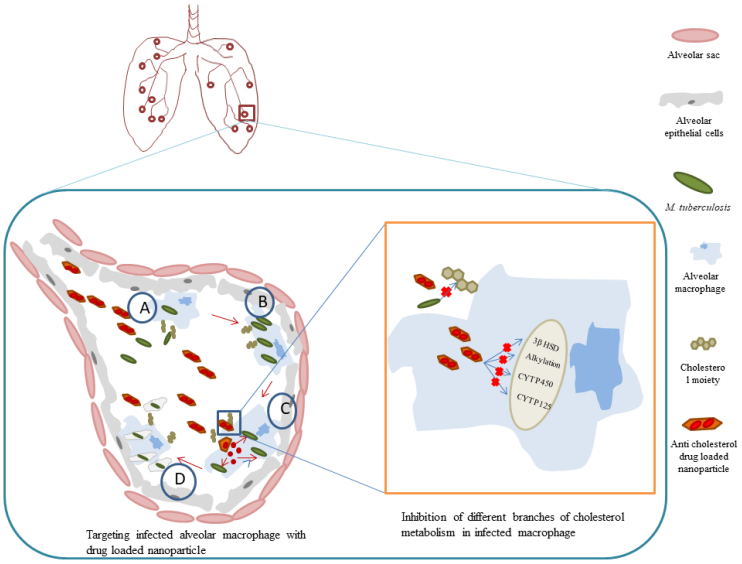

Figure 2: Treatment by anti-cholesterol drug encapsulated nanoparticle: After infection into the host cell, *M. tuberculosis* reside into alveolar macrophage. **(a):** *M. tuberculosis* utilizes host cholesterol for attachment and entry into the macrophages; **(b):** A bacillus has the advantage to replicate inside macrophage without any interference of the host immune system; **(c):** Mannosylated or specific biomarker coated nanoparticle target infected macrophage and can hinder with the metabolism of cholesterol by inhibiting various enzymes of cholesterol metabolic pathway; **(d):** Non degradation of cholesterol metabolism could lead to accumulation of toxic metabolic intermediate which can lead to bacterial death and apoptosis.

Table 3: Anti-cholesterol drugs.

S.No.	Anti-Cholesterol Drug	Target	Reference
1.	Azasteroids	Inhibit 3β HSD enzyme that catalyzes conversion of cholesterol to 4-en-3-one	34
2.	Clotrimazole and econazole	Bind with CYP450s and inhibit its function	35
3.	3β-hydroxysterol-(25R)-cholest-5-en-3β,16β,26-triol	Inhibit alkylation of cholesterol side chain	36
4.	V-13-011503 and V-13-012725	Inhibit the function of HsaAB that converts 3-HAS to 3,4-DHSA (3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione) in the catabolism of the A/B rings of cholesterol	37
5.	3-chlorocatechol	Inhibitor of extradiol dioxygenase	38
6.	Phenylmethylsulfonyl fluoride and 3,4-dichloroisocoumarin	Modify the function of HsaD which catalyzes the hydrolysis of C-C bond	39
7.	20,60-dichloro-DHB and 4-chloro-3-dihydroxy-6-methyl-7,8-dihydro-10-Cl-stilbene	Inactivates HsaC Which is type I iron-dependent extradiol dioxygenase that cleaves 2,3-dihydroxybiphenyl(2,3-DHB)	40

drug development there are other branches also which are important for maintaining the intactness of the bacilli. One of them is the secretory transportation system employed by *M. tuberculosis.* Studies prove that twin-arginine transporter system (TAT) is an important translocator responsible for transportation of folded proteins to its destined location. *M. smegmatis* that is deficient in tatA and tatC are unable to grow on agar medium, defective against beta-lactamases and show a hypersensitive pattern for Sodium Dodecyl Sulfate (SDS). Excretory system (ESX) is the major secretory system composed of several proteins that reside in the mycobacterial cytoplasmic membrane and carrying the exportation of cytoplasmic molecules to the extracellular surroundings. These proteins are the major culprit in causing drug-resistant TB [44,45].

M. tuberculosis is the reason behind approx 1.5 million lives for each subsequent year, making it the main source of mortality from prominent bacterial infections. *M. tuberculosis* is almost constrained in among 33% of the total population, 90% of whom show symptom less inactive diseases that speak to a subclinical or latent pool of pathogenic *M. tuberculosis* strain and convoluted tuberculosis (TB) control methodologies [46]. The prevalence of TB is expected to a limited extent to this capacity to set up unending disease, alongside the deficiency of present treatments [47]. The WHO has proclaimed TB a worldwide threat and declared emergency against it. The basic fundamental aspect behind its residence in host cellular environment is dependent on inability of the host immune system to rescue themselves and inhibition of the biogenesis of phagosome lysosome complex known as phagolysosomes.

Cholesterol has a significant perspective on the infectivity and pathogenicity of *M. tuberculosis*. Host cholesterol has been appeared to encourage the survival of mycobacteria into macrophages [48]. Moreover, *M. tuberculosis* is able to utilize cholesterol as a carbon source. The cholesterol metabolism pathway in mycobacteria has been proposed in view of the assurance of the qualities associated with cholesterol catabolism in the *Rhodococcus* species. The majority of these qualities in *M. tuberculosis* have been observed to be under the influence of kstR regulons, which encode a TetR-like transcriptional repressor [49,50]. A few qualities in the kstR cholesterol regulons are incited in macrophages or are basic for disease, accentuating the job of cholesterol catabolism in intracellular survival.

This implausible width of multiplicity among different nanoparticle purposes has been established successfully for an extensive range of treatments, including multiple cancer chemotherapies, ARVs, and even suntan lotion. For the perspective of TB, it already grants considerable improvement; amongst which the many advantages are as increased carrier capacity, reduced degradation in the bowels, improved stability, lesser cytotoxicity and the ability to cater to both a hydrophobic and hydrophilic environment [51,52].

It can provide both sustained and targeted drug delivery and could deliver drugs either to plasma of blood or to the particular organ tissue. In this way, this mechanism gains success to establish direct contact between the pathogen and drug and no other part will be affected. Therefore it is of great advantage over commercial drug and normal drug delivery medication. Although we only emphasize to target cholesterol metabolism of *M. tuberculosis* by several anti-cholesterol medications, there are many other aspects that could be and should be targeted by this nanoparticle approach to obtain a successful and better way achievement in the way of eradication of TB [53-55].

One of the important targets to achieve this treatment is to target the vitamin D system of *M. tuberculosis* as it had been proved that it is one of the major pathways running in this pathogenic bacterium. Retinoic acid and vitamin D3 are also important in downregulating the growth and survival of *M. tuberculosis.* Vitamin A and vitamin D are major vitamins that have involved in protection of host against infection. Earlier studies show that retinoic acid and vitamin D3 together suppress the expression of TACO protein in a dose-dependent manner which therefore also can halt mycobacterial entry to the host cell [45]. Transportation of accessory retinoic acid and vitamin D3 along with nanoparticles could be beneficial to suppress the survival of *M. tuberculosis*. By now we know that *M. tuberculosis* is highly aerobic bacteria so we can develop a drug or by the help of nanoparticle we can take away the free oxygen molecules which are needed by *M. tuberculosis* in order to survive. We intend to make our drugs more specific to target the pathogenic strain which is least harmful to the body and is more effective against the pathogen and treat the disease in as much less time as possible. This methodology of targeting the cholesterol utilizing arm of the bacterium which is the major carbon source will definitely raise an effective anti TB drug. This is because inhibition or hindrance in this pathway would lead to accumulation of toxic metabolites that ultimately lead to bacterial death. So studying in this direction would absolutely a beneficial side for social benefit in terms of getting rid of this disease.

Discussion

Drug delivery is a major concern in case of tuberculosis due to the persistent nature of the bacterium inside granuloma without even any information to host immune system. TB is a global disease caused by the bacteria *M. tuberculosis.* The genome of *M. tuberculosis* is studied up to a great extent but still there are some complexities which are needed to be understood as it shows number of variations from normal pathways on exposure to different drugs and steroids and the resistant nature of the bacterium [56-58]. *Mycobacterium*

when residing in the granuloma in host utilizes host cholesterol as their nutrient and main lipid content. Cholesterol, therefore, is one of the major reasons behind this malady because it maintains its growth inside the granuloma.

Conclusion

This review emphasizes the importance of cholesterol in mycobacterial survival and growth and nanoparticle-mediated delivery of anti-cholesterol drugs to cease the uptake and utilization of host cholesterol by *M. tuberculosis.* Anti-cholesterol drugs would be loaded onto a nanoparticle which would be designed with specific mannose biomarkers that specifically traverses through host inhalatory system to reach alveolar macrophage containing bacilli. Target specific delivery is beneficial in several manners as in lower dosage, lesser cytotoxicity, and effective treatment.

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