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Review title

Impact of Statins on Mycobacteria Drug-Resilient Biofim

Purva Vaijanath Puskar[1], Asst. Prof. Khalid K.U[1], Prof. Shyamlila Bawage[3]

UG Student, Latur college of pharmacy, Hasegaon[1]
Department of pharmaceutics, Latur college of pharmacy, Hasegaon[2]
Department of pharmacognosy, Latatur pharmacy college, Hasegaon [3]

ABSTRACT:

Tuberculosis is one of the top ten causes of death in the world. Tuberculosis air pollution caused by M. tuberculosis affects any part of the body and usually the lungs. M TB. has a waxy coating that sticks to its cells mainly due to the presence of mycolic acid. Since then, several mycobacterial species, including M. tuberculosis, has been identified as a biofilm drug that is genetically tolerant. Bacterium biofilm films are a major concern for global health because of their ability to tolerate antibiotics, the immune system involved. TB M. needs cholesterol to get dirty and pass on to the person who has it. Targeted treatment is a new and promising way to treat TB. Statins inhibitors of HMG-CoA reductase, an enzyme that restricts the level of cholesterol biosynthesis pathway, which causes the conversion of HMG-CoA reductase to mevalonate and has a pleiotropic effect, including the proliferation of immunomodulatory, anti-inflammatory effects. and antimicrobial. It is calculated that cells treated with statins are highly resistant to M. tuberculosis disease. The point of this review is to integrate the logical information available from biofim development with Mycobacteria tuberculosis and the effect of statins on M. tuberculosis and biofilms of microorganisms.

Keywords: Mycobacterium tuberculosis; Cholesterol; Statins; Biofilms

Introduction

Tuberculosis (TB) remains a major health threat, especially in developing countries. These diseases are identified by various names such as king evil, phthisis, tapedic, white plague, Consumption disease and more. It is caused by bacillus Mycobacterium tuberculosis which is spread when people with TB expel the virus from the air; for example, by coughing, sneezing. It mainly affects the lungs (TB of the lungs) but may also affect different areas (TB outside the lungs). It is estimated that 1.2 million people died of TB among people living with HIV (- ve) in 2018, and 251,000 died of HIV (+ ve) people. Geographically, the majority of TB cases in 2018 were in the WHO regions of Southeast Asia (44%), Africa (24%) and the Western Pacific (18%), with small numbers in the Eastern Mediterranean (8%), United States . . (3%) and Europe (3%) [1].

Currently TB treatment involves the use of many drugs especially isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol over a period of 6-9 months. Such treatment is discontinued as a result of non-compliance and the presence of multidrug-resistant TB (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB). Multidrug resistant (MDR) is the most resistant to both isoniazid and rifampicin. Extensive drug resistance (XDR) is resistant to any fluoroquinolone, as well as any one-third drug of the second injection (capreomycin, kanamycin and amikacin), in addition to drug resistance. Drug-resistant tuberculosis (TB) is a formidable obstacle to effective TB care and global expectations. In 2018, almost a large portion of 1,000,000 young people rifampicin - drug-resistant TB was tested. Drug resistance to TB is caused by changes in M. tuberculosis which makes the anti-TB drugs less effective against mutant tubercle bacilli. It may arise as a result of improper use of antibiotics in TB patients at risk of drug abuse [2].

The cell wall of M. tuberculosis contains a large proportion of lipids, and the central part of its gene comprises proteins to be synthesized. It has been shown that mycobacteria bind and use cholesterol as a source of carbon and a combination of other virulence components. In mycobacteria, stress and quorum sensitivity lead to the formation of biofilm [3].

Biofilm is an unexpected structure of a microbiome with columns of various viruses or single cell types in a group; holding his face. These cells are embedded in an extracellular polymeric substance, a matrix normally composed of eDNA, proteins and polysaccharides, which exhibit high resistance to antibiotics. About 80% of chronic and recurrent bacterial infections in the human body are caused by a bacterial film. M. tuberculosis can similarly form a biofilm film that undergoes casseous necrosis over time and the development of a hole in the lung tissue. It was recommended that mycolic acids, such as DNA, be essential for the development and regulation of M. tuberculosis biofilms. Biofilm films provide protection against microorganisms not only from proper pH, osmolarity, deficiency, mechanical strength and shear but also prevent the entry of antibacterial and immune cells. In the structure of biofilm, micro-organisms are highly resistant to various antimicrobials. In line with this, biofilm drug tolerance has become a major obstacle to the effective management and treatment of TB. In a situation where drug resistance is rapidly spreading. Comprehensive treatment is a new and promising way to treat tuberculosis [4]. Control of explicit immune responses, including those involving inflammation and immunopathology, can limit mycobacterial infection and pathology, both in cell culture and in animal models.

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) provide an alternative to home remedies, such as widely used drugs that have been shown to be safe and effective. Using targeted drugs as an adjunct to standard anti-TB treatment can also reduce the duration of the disease and inevitably lead to a decrease in recurrence episodes [5]. These drugs, which include rapamycin, metformin, valproic acid, ibuprofen, and statins, have similarly been shown to influence the growth of mycobacteria in vitro cells.

Statins, a class of drugs used to lower blood cholesterol levels. However, it was noted that Statins has several side effects in addition to its well-known anti-hyperlipidemic action, including antimicrobial, immunomodulatory, anti-oxidative and anticoagulant effects and more; which have been associated with better outcomes in the treatment of fewer preventable diseases. All observations, statins interactions and treatment of multiple drugs effectively reduce mycobacterial activity [7]. It was shown that statins have the potential to cause variable levels of antibacterial activity against S. aureus, B. pumilus, S pneumoniae, M catarrhalis P. aeruginosa, S. Enteric, Acinetobacter, baumannii, proteus mirabilis, enterobacter cloacae, M. tuberculosis etc .; and various pathogens. Simvastatin, used in the treatment of atherosclerotic cardiovascular disease and hypercholesterolemia, also showed anti-bacterial activity in monotherapy against M. tuberculosis [8] [9].
Gupta and Kumar (2019) and numerous authors evaluated the MIC estimation of different statin drugs and presumed that Simvastatin, Atorvastatin, and Fluvastatin could be created as likely antimicrobial agents [10].It was examined simvastatin demonstrated antimicrobial activity against S. Aureus, Pseudomonas aeruginosa, Escherichia coli and Enterococcus faecalis diminishing their biofilm development and viability[40].

Antifungal activity of certain statins against various fungal species has been additionally detailed as simvastatin inhibit planktonic cells and biofilms of Candida and Cryptococcus species. These proof of statins against biofilm make guarantee have an extraordinary potential as a compelling antibacterial tool for biofilms of M. Tuberculosis also. the extent of statin treatment has extended with the emergence of evidence suggesting that due to pleiotropic impacts of statins that are not legitimately connected with their regulation of cholesterol levels, they may end up being valuable for treating various number of diseases include multiple sclerosis (MS), inflammatory bowel diseases (IBDs), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), chronic obstructive pneumonic ailment (COPD), malignant growth, strokes, Parkinson's and Alzheimer's diseases, bacterial infections, and HIV and so forth; [11].

Mycobacterium tuberculosis and pathogenisis:

Mycobacterium tuberculosis is aspecies of pathogenic microscopic organisms in the family Mycobacteriaceae and the causative agent of Tuberculosis. First found in 1882 by Robert Koch. They are aerobic, non–spore-forming, nonmotile, facultative, bended intracellular rods estimating 0.2-0.5 µm by 2-4 µm. It has a complex thick waxy cell wall because of its high lipid content; this goes about as an obstruction to anti-microbials and is resistant to lysosomal enzymes, empowering the microorganisms to make due inside macrophages in the body. This waxy layer additionally shields the bacilli from drying out so they may survive many months in the air and dust as long as it is dull. Mycolic acid instigates the aggregation of cholesterol inside peritoneal and alveolar macrophages. Macrophages containing cholesterol and lipid beads look like foamy macrophage derivatives saw in tuberculous granulomas[49].
Fundamentally Infection happens when an individual breathes in droplet nuclei containing tubercle bacilli that arrive at the alveoli of the lungs. There is an almost certain danger of transmission when roughly one to 10 bacilli are scattered through the air. These tubercle bacilli are ingested by alveolar macrophages; most of these bacilli are obliterated or inhibited. A modest number may increase intracellularly and are delivered when the macrophages die[12]. In the event that alive, these bacilli may spread by lymphatic channels or through the bloodstream to more inaccessible tissues and organs (counting lymph nodes, apex of the lung, kidneys, cerebrum, and bone).

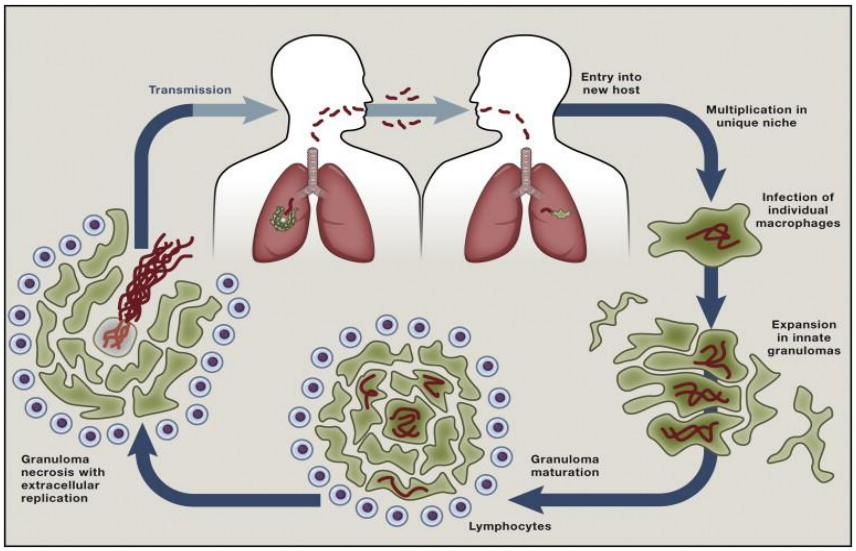

Figure no.1 Pathogenisis of *M.tuberculosis*

The alveolar macrophages, after entry of *M. tuberculosis*, produce inflammatory cytokines and chemokines that fill in as a signal for infection. The monocytes, neutrophils, and lymphocytes relocate to the central site of disease, yet they can't able to kill the bacteria efficiently. During this time, the bacilli resist the bactericidal mechanisms of the macrophage (phagolysosome) by preventing phagosome-lysosome fusion, duplicate in the phagosome, and cause macrophage necrosis[13].

The association of *M. tuberculosis* ligand with TLRs in the end brings in activation of nuclear transcription factor (NF)- κB and production of proinflammatory cytokines, for example, tumor necrosis factor (TNF)- α, interleukin (IL)- 1, IL-12, chemokines, and nitric oxide through either myeloid differentiation primary response protein 88 (MyD88)- dependant or MyD88- independent pathway[14][15].The IFN-γ is the key cytokine for a defensive immune response against *M. tuberculosis*. Humans and mice defective in IFN-γ or IFN-γ receptor genes are more susceptible to *M. tuberculosis* contamination[16].

Drug tolerance by biofilm formation in *M.tuberculosis*

Diseases of *M. tuberculosis* are regularly asymptomatic, chronic in a clinically symptomatic state and exceptionally unmanageable to antibiotics[17].Although molecular mechanisms underlying persistence of the microorganism against the host immune system and chemotherapy remain unclear. Ever-mounting proof shows that the endurance of numerous ecological and pathogenic microbial species against anti-microbials is affected by their capacity to develop as surface-related multicellular communitiescalled biofilms. As of late, a few mycobacterial species, including *M. tuberculosis*, have been found to frame drug-tolerant biofilms in vitro through hereditarily controlled mechanisms[18].

The primary reports originated from cases of tuberculosis disease related with clinical biomaterial, prosthetic joints specifically. Further analyses have demonstrated a decrease in the action of anti- tuberculous medications against tuberculosis biofilms. These revelations offer ascent to enthusiasm for biofilm forming mechanisms as an expected objective for new treatments against tuberculosis. It has been proposed that the significance of biofilms in this infection is because of their cooperation during the process of casseous necrosis and cavitation development in lung tissue, a site where *M. tuberculosis* could form a biofilm[19].

Biofilm development is a significant factor in antimicrobial resistance. It manages numerous bacterial species security against antibiotics ordinarily active against similar microscopic organisms in the planktonic state[20].Later investigations described *M. tuberculosis* forming "pellicles" in fluid media, with pictures very like what in current times are depicted as biofilms[21].Coordination inside the biofilm through cell-to-cell communicationcalled quorum sensing (QS) in which aggregation of signaling particles in extracellular climate promptsregulation of the specific genes expression. Preliminary evidence proposes that biofilms could might be an in vivo way of life of *M. tuberculosis*, adding to their persistence against antibiotics. In an obsessive report by Lenaerts et al., the enduring population of the microorganism after medication treatment of infected guinea pigs was discovered to be in microcolonies of bacteria situated around the acellular edge in the granulomas[22].

Biofilm occupant bacteria show 100-1000 folds higher minimal inhibitory concentration (MIC) when contrasted with planktonic microbes making their treatment a difficult assignment. The ability of Mycobacterium to form biofilms described in 1989.It is accepted that the extracellular polymeric substance (EPS) could go about as an obstruction for antibiotic entrance and along these lines may add to thedrug tolerance saw in biofilms. EPS gives mechanical stability to biofilms through physiochemical interactions that include electrostatic powers, hydrogen bonds and van der Waals interactions. In spite of the fact that the composition of EPS changes essentially among various bacterial species, extracellular polysaccharides, proteins and lipids stay as the key segments of EPS.A number of Mycobacterial species are known to form biofilms including *Mycobacterium tuberculosis* (Mtb), *Mycobacterium smegmatis* (Msm), *Mycobacterium avium*, *Mycobacterium marinum* and *Mycobacterium ulcerans*[23].It has become certain that biofilm-developed cells express properties particular from planktonic cells, one of which is an increase resistance from antimicrobial agents.Notwithstanding, biofilms could shield the pathogenic mycobacterial species from the immune system of the host and could assist bacteria with persist during treatment with antibiotics. A few mycobacteria can build up these structures on surfaces, yet additionally on the in air-media interface[24].

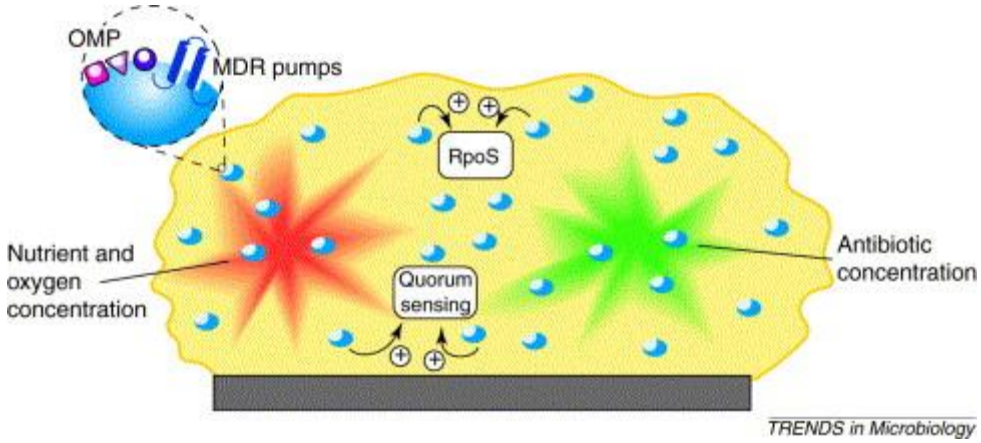

Figure no.2 Drug resistance in biofilms

This wonder might be clarified by the diverse composition of the extracellular matrix of the biofilm and the interesting attributes of mycobacterial cell wall, particularly the presence of high lipid levels. As the data accumulated about biofilm formation in mycobacteria it ensure that anti-lipids drug (Statins) have capacity to prevent biofilms.The biofilm development happens through a progression of steps including the initial attachement of the bacterial cells to substratum which is followed by the accumulation of the cells and irreversible authoritative. This progression is followed by maturation of the biofilm cells which is formed by layering of the aggregates, which after arriving at an extreme thickness begins to scatter just to beginaggregating at a new site.

Estimated that after changes can happen in biofilm-grown bacteria :

- Induction of the general stress response (an rpoS dependent process in Gram-negative bacteria);
- Increasing expression of multiple drug resistance (MDR) pumps;
- Activating quorum sensing systems; and
- Changing profiles of outer membrane proteins (OMP)

Host directed therapy

Tuberculosis is a significant reason of morbidity and mortality around the world. The current treatment against tuberculosis depends on the organization of a combination of antimicrobials for six months. be that as it may, the lenghty treatment and its adverse impacts favor poor adherence, disappointment, and the development of resistance. In spite of clinical cure, around half of treated patients have permanent lung damage because of abundance inflammation caused by this disease[25].

These realities underscore the need for the advancement of new or potentially improved TB treatment strategies. Host-directed therapies are a generally new and promising way to deal with treatment of tuberculosis. The term HDTs is utilized to characterize treatment choices with small molecules or biologics that can give an antimicrobial or advantageous impact.Modulation of specific host immune pathways, including those that impact inflammation and immunopathology, can restrict mycobacterial infection and pathology, both in cell culture and in animal models.Drugs focusing host processesmay generally stay away from the development of bacterial anti-microbial resistance, a significant general wellbeing worry for tuberculosis. Subsequently, it is fundamental to discover restorative agents with the possibility to shorten treatment time and in the long run, with the ability to fortify the immune response against *M. tuberculosis*.

Host-directed therapy (HDT) is an emerging approach in the field of anti-infectives;

- Host-directed therapy (HDT) is a novel proposal in the field of anti-infectives for overcoming antimicrobial resistance.
- HDT aims to interfere with host cell factors that are required by a pathogen for replication or persistence, to enhance protective immune responses against a pathogen, to reduce intensify inflammation and to balance immune reactivity at sites of pathology.
- In Tuberculosis, HDT aims to enhance the antimicrobial activities of phagocytes through phagosomal maturation, autophagy and antimicrobial peptides. HDTs also curtail inflammation through involvement with soluble (such as eicosanoids or cytokines) or cellular (co-stimulatory molecules) factors and modulate granulomas to allow the entry of antimicrobials or to restrict tissue damage.
- The goals of HDTs against TB may be several and may include shortening the course of treatment; reducing the number of agents required in combination drug therapy, particularly when toxicity is a concern; improving the efficacy of regimens against drug-resistant TB, which are known to be usually lower than the standard one; preserving lung function in a context of extensive tissue damage.[26]

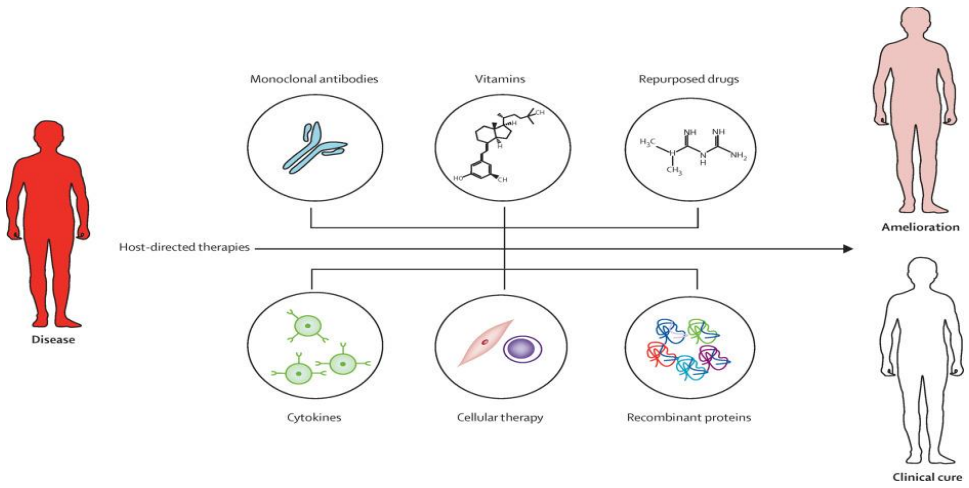

Figure no.3 Classes of HDT

Statinsas repurposed drugs

Here we discussed about repurposed drug as HDT, Repurposing drugs includes finding drug target interactions of established drug treatments, with a aim to utilize them to treat various infections[27].Repurposing and restoration of the drugs are the new trends/choices to battle these decline circumstances of tuberculosis in the anti-microbials resistance time or in the circumstance of worldwide crisis. Numerous medications have been effectively repurposed to date. In a Nigerian preliminary examination on patients of HIV-MDR-TB coinfetion, proficiency of MDR-TB treatment by trimethoprim/sulfamethoxazole TMP/SMX confirmed a significantly shorter time to sputum change in these patients. Sulfadiazine is an anti-leprosy drug which is repurposed in the treatment of MDR-TB and XDR-TB. Suggested that sulfadiazine routine is protected and successful against MDR-TB and TDR-TB treatment. Clofazimine (CZM) is one of the repurposed molecules, has been at first utilized as an anti-leprosy drug since a large portion of the century. It was as of late repurposed for dealing with the treatment of MDRTB. It was accounted for that metformin is a promising candidate as repurposed drug for improving the compelling treatment of tuberculosis.

These types of drugs, including rapamycin, metformin, valproic acid, ibuprofen, and statins, have also been shown to affect the growth of mycobacteria in infected cells in *in vitro* studies .By given their pleiotropic activities, statins are strong andmost potential candidatesto be repurposed as novel antimicrobial agents.[29]

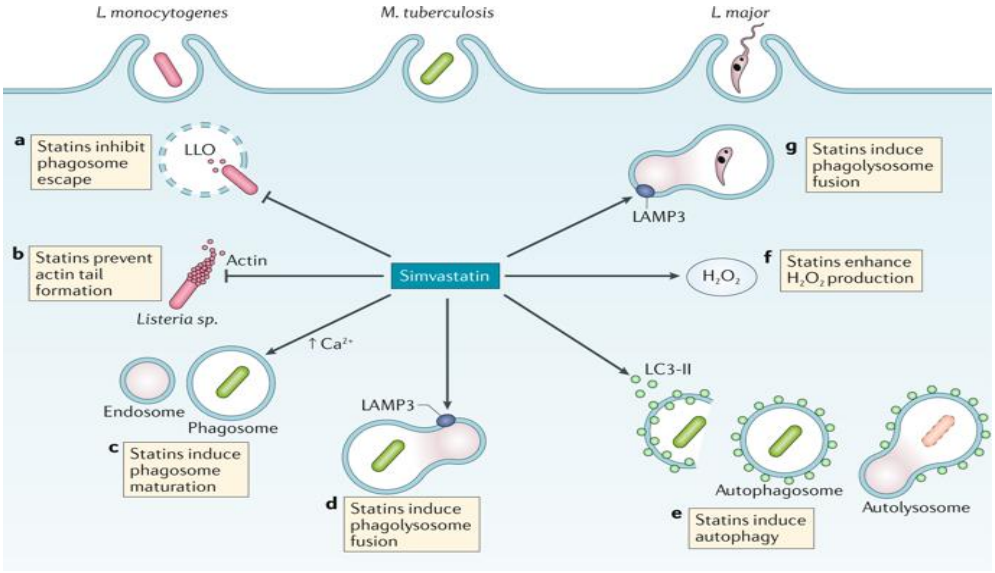

Figure no. 4 Mechanism of statins on M. tuberculosis

Studies have shown that cholesterol is crucial for the uptake of mycobacteria by macrophages, and it has been found that assemble at the site of *M. tuberculosis* entry.[48]Moreover, cholesterol depletion overcomes the phagosome maturation block experienced by *Mycobacterium tuberculosis* infected macrophages.Statins bring their cholesterol-lowering effect by binding tothe active site of 3-hydroxy-3-methylglutaryl-coenzyme A(HMG-CoA) reductase (HMGR), a rate-limiting enzyme involvedin cholesterol biosynthesis .[30]HMGRis an essential part ofthe mevalonate pathway, which not only is essential for cholesterolbiosynthesis but also contributes to the production of isoprenoids,lipid compounds that are essential for cell signaling andstructure. As well as inhibition of cholesterol, statins have alsobeen found to have a number of cholesterol-independent, socalled"pleiotropic" effects (anti-inflammatory, anti- bacterial, immunomodulatory, and anti-cancer effectson host cells, and these effects are well characterized).

Pleiotropic effect of statins

Statins inhibit cell proliferation by inhibiting Ras and Rho proteins and stabilizing the levels of cell cycle proteins (p21 and p27). Furthermore, statins induce apoptosis by upregulating proapoptotic proteins (ie, Bax) and downregulating antiapoptotic proteins (ie, Bcl-2). [31]statins induce the phagocytic activity of macrophage J774. It has also been reported that they act as inhibitors of the expression of MHCII induced by IFN-γ in primary endothelial cells, monocytes, and humanmacrophages, which in turn inhibits the activation of T lymphocytes.The treatment of mononuclear cells with fluvastatin produced the discrete activation of caspase 1 and moderated the secretion of IL- 1β, IL-18, and IFN-γ.It has also been demonstrated that statins upregulate IL-10 in the serum of patients with acute coronary syndrome. Another study showed that the *in vitro* treatment of mononuclear cells with atorvastatin increases the number of NK and NKT cells in peripheral venous blood.It has also been shown that simvastatin therapy in patients with hypercholesterolemia for six months increases the iNKT cells in peripheral venous blood. Other studies show that statins can induce apoptosis in human cells from tumors through the inhibition ofRas signaling pathways. Statins also promote autophagy through the activation of the AMPK-TOR signaling pathway in cells from rhabdomyosarcoma. Treatment with lovastatin increases the expression of Rab7 mRNA by decreasing the synthesis of isoprenyl groups and promoting phagosome maturation.Importantly, statins also impact vascular and immune functions via altered NO signaling leading to improved vascular function, inhibition of leukocyte chemotaxis, and downregulation of leukocyte adhesion and migration at the vascular

wall.[32]

Emerging reports have indicated the pleiotropic effects(apart from cholesterol-lowering properties) of statin drugssuch as inflammation reduction , immunomodulation, and as an anti-bacterial.Recently, various articles have also reported theantimicrobial effects and immunomodulation of statin drugs against mycobacterium tuberculosis.

***In-vitro and in-vivo*effectof statins on *M.tuberculosis*and other Infectious Diseases**

The first study to highlight the potential effects of statins on *M. tb* was conducted20 years ago by Montero *et al*., where it was observed that fluvastatin modulates the release oftype 1 T helper (Th1) and type 2 T helper (Th2) cytokines and consequently activates caspase-1 or leads to the secretion of interleukin (IL)-1β, IL-18 and interferon gamma (IFNγ).[30]In 2014Parihar *et al*.found that mononuclear cells and monocyte-derived macrophages from patients with familial hypercholesterolemia who had received statin therapy for at least six months were more resistant to infection with M. tuberculosis. In the same study, bovine bone marrow-derived macrophages (BMDMs) were treated with 50μM of simvastatin and were infected with M. TB for four hours at 37 ℃. The results depicted asignificant decrease in bacterial growth without alterations in cellular viability.Metabolic rescue experiments demonstrated that statins reduce membrane cholesterol levels, particularly by the mevalonate-isoprenoid arm of the sterol pathway. This promoted phagosomal maturation (EEA-1/Lamp-3) and autophagy (LC3-II), as shown by confocal microscopy and Western blot in macrophages. [8]

Guerra-De-Blas *et al*. analyzed the effects of simvastatin on the treatment of *M.tb*infection. Observed thatsimvastatin decreased the growth of *M. tuberculosis* in PBMCs, increased the proportion of NKT cells in culture, increased the expression of co-stimulatory molecules in monocytes, promoted the secretion of the cytokines IL-1β and IL-12p70, and activated apoptosis and autophagy in monocytes, resulting in a significant depletion in bacterial load. Also observed an increase in IL-10 production. simvastatin activates several immune mechanisms that favor the containment of *M. tuberculosis* infection, which providing relevant evidence to consider statins as candidates for host-directed therapy.[33]

Recently, Gupta and Kumar(2019) have reviewed the MIC value of various statin drugs and concluded that Simvastatin, Atorvastatin, and Fluvastatin could be developed as potentialantimicrobial agents.[10]Lobato *et al.* investigated that statin increase rifampin mycobactericidal activity for *M. tuberculosis*, both statins (2 μM atorvastatin and 2 μM simvastatin) reduced the viability of the mycobacteria by approximately 75% and also showed an additive effect with rifampicin (1 μg/mL rifampicin plus 0.2 μM atorvastatin or 0.2 μM simvastatin. However, for *M. leprae* strain, they inoculated 1×10 4 live *M. leprae* strains into plantar pads of each shepherdmodel of BALB/c mice and noticed that only atorvastatin showed an additional effect with RIF.[34]

Dutta et al. (2016)confirmed that simvastatin therapy as an adjuvant to standard treatment reduced the time to obtain a negative culture in BALB/c mice infected with *M. tuberculosis* H37Rv by aerosols. They also evaluated the relapse rates in mice treated with simvastatin (60 mg/kg) for 2.5, 3.5, and 4.5 months. Relapse was evaluated three months after stopping treatment. The results showed that treatment with anti-TB drugs plus simvastatin reduced the percentage of relapses by 50% compared with treatment with only anti-TB drugs.[7]
Dutta *et al.* conducted another study in 2019 on female C3HeB/FeJ mice, in which Pravastatin (7.8 μM), simvastatin (0.2 μM) and fluvastatin (0.032 μM) enhanced the activity of first-line anti-TB drugs (0.006 μM INH plus 0.0055 μM RIF and 81.23 μM PZA). However, atorvastatin and mevastatin showed no effect on mycobacterial growth at non-toxic doses. Among all statins, pravastatin exhibited the most potent adjunctive activity with the least toxicity by modulating phagosomal maturation characteristics in THP-1 macrophages.[50]

Skerry *et al*.simvastatin reduces bacterial load in an *in vitro* macrophage model and enhances the effects of standard treatment in chronically infected BALB/c mice. standard oral regimen of rifampicin (10 mg/kg), isoniazid (10 mg/kg) and pyrazinamide (150 mg/kg) given five times weekly, the addition of 25 mg/kg simvastatin enhanced bacillary killing, reducing the number of lung cfu by an additional 1 $\log_{10}$ at Day 28 ($P < 0.01$) and by a further 1.25 $\log_{10}$ at Day 56 ($P < 0.01$).[9]Pravastatin modulated phagosomal maturation characteristics in macrophages, phenocopying macrophage activation, and exhibited potent adjunctive activity in the standard mouse model ofTB chemotherapy and in a mouse model of human-like necrotic TB lung granulomas.[35]Several retrospective cohorts and population-based case-control studies have also highlighted theeffects of statin on the risk of developing TB. [52][53]

Other than this Simvastatin showed a significant antimicrobial effect against Methicillin-sensitive staphylococcus aureus (MSSA) MIC 29.2 mg/L and to a lesser extent against methicillin-resistant staphylococcus aureus (MRSA) MIC 74.9 mg/L.[36] [37] The MIC-value for simvastatin against *S pneumoniae* and *M catarrhalis* was determined 15 mg/mL (36 mmol/L).[39] Statins also induce tumour-specific apoptosis through mitochondrial apoptotic signaling pathways, which are activated by the suppression of mevalonate or geranylgeranyl pyrophosphate (GGPP) biosynthesis and It has been observed that lovastatin interferes with the replication of hepatitis C virus RNA through the inhibition of geranylgeranylation protein of the host. Statins also inhibit the assembly of dengue virus virions through a mechanism independent of cholesterol levels. It have also shown antiviral effects on cytomegalovirus, the Epstein-Barr virus and HIV infection.[38]Bergman *et al.* found a MIC value of 15.6 μg/mL for simvastatin against Streptococcus pneumonia. [39] Ribeiro *et al*., 2017 *In vivo*, atorvastatin plus fluconazole increased the survival of mice and reduced the burden of *C. gattii* in the lungs and brain. Authors demonstrated the in vivo efficacy of topical Simvastatin against microbial infection in BALB/c mice [47]

These studies have demonstrated that cholesterol plays a carping role in the pathogenesis of *M. tuberculosis* infections and other infectious diseases in several ways, including the entrance of *M. tuberculosis* into host macrophages,phagosome formation,the arrest ofphagosomal maturation in M. tuberculosis-containing phagosomes,and the energy utilisation of intracellular *M. tuberculosis*.Statins can stop TB infection by reducingmacrophage

cholesteroland phagocytosis.Statin can alsoreduce cholesterol levels within phagosomal membranes andcounteract the *M. tuberculosis* induced inhibition of phagosomalmaturation to further host-induced autophagy in humanmacrophages and experimental mouse models.Thus, statinsmight exert a protective effect against TB infection.

Anti-biofilmeffects of statin

Graziano *et al*. 2015Simvastatin has shown pronounced antimicrobial activity against *S. aureus* biofilmsby reducing their formation and viability.Simvastatin'sMIC was evaluated 15.65 μg/mL for S. aureus 29213 and 31.25 μg/mL for the other strains of S.aureus.[40]In established biofilm simvastatin decreased *P. gingivalis* counts by up to more than 1′000-fold[41].*In vitro* studies show that noncandidacidal concentrations of simvastatin (1, 2.5, or 5 μM) inhibit biofilm production *in vitro* by laboratory and clinical isolates of *C. albicans* in both microscopic and spectrophotometric assays.[42]

In other study, Simvastatin showed inhibitory effect against *Candida* spp. and*Cryptococcus* spp with MIC values ranging from 15.6 to 1000 mg L^{-1} and from 62.5 to 1000 mg L^{-1}, respectively. The combination of simvastatin with itraconazole and fluconazole showed synergism against *Candida*spp. and *Cryptococcus* spp., while the combination of simvastatin with amphotericin B was synergistic only against*Cryptococcus* spp. Concerning the biofilm assays, simvastatin was able to inhibit both growing biofilm and mature biofilm of*Candida* spp. and *Cryptococcus* spp.[43]Statins also inhibit the formation of biofilms of *Candida albicans* [44]and, in *C. glabrata*, reduce ergosterol levels, inhibit their growth, and cause the loss of mtDNA.[45][46]These studies show that statins slow the growth of some microorganisms, including some resistant bacteria, and also show that they can interfere with biofilm formation.

Conclusion

Emergence and spreading of anitibiotics resistant M. tuberculosis due to biofilm formation have worsened the current situation across the globe.In this review we have discussed about pathogenesis of *M.tuberculosis*, drug tolerance by biofilm formation and statins provided protective effects against*M.tuberculosis*through immunomodulation and anti-microbial activity. the most convincing evidence is statins showed anti- biofilm action towards various gram-positive and gram-negative bacteria and on some viruses and fungi . These effects have clinical relevance because within the biofilm, bacteria are protected against the action of antibodies, the attack of phagocytic cells, and the effect of antimicrobials. Therefore, it is very important to note that statins have the potential to inhibit the growth of resistant bacteria and interfere with the biofilm formation process.It is believed that the extracellular polymeric substance (EPS) could act as a barrier for antibiotic penetration in biofilm.The Extracellular matrix of *M.tuberculosis* Biofilm contains the high amount of lipids, therefore statin have ability to prevent this barrier . Such multidisciplinary communication can hope that prevention and inhibition of biofilms of *M.tuberculosis* can be achieved through use of statins innear future which might be help to achieve the goal of TB free world declared by WHO.

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