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Metformin for Tuberculosis Infection

*Bernadette Dian Novita, Ari Christy Mulyono
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Abstract

Tuberculosis, caused by *Mycobacterium tuberculosis* (M.tb), remains the biggest infection burden in the world. Rifampin (RIF) and Isoniazid (INH) are the most effective antibiotics for killing M.tb. However, the resistance rate of rifampin and INH are high and lead to almost 35% treatment failure. Metformin enhanced anti tuberculosis efficacy in killing *M. tuberculosis* through several mechanism, firstly through autophagia mechanism and secondly by activating superoxide dismutase (SOD). Metformin activated mTOR and AMPK then induced more effective autophagy against M.tb. Superoxide Dismutase (SOD) is an enzyme produced in the host's antioxidant defense system. SOD neutralizes reactive oxygen species (ROS) that excessively produced during phagocytosis process against M.tb. Excessive production of ROS associated with Th1 overactivation and leads into macrophage activity inhibition and excessive tissue damage. Metformin has ability in improving SOD level during inflammation.

Keywords: metformin, tuberculosis, autophagia, anti tuberculosis enhancement

1. Introduction

Metformin is a biguanide salt hydrochloride consists of a molecular component of $C_4H_{11}N_5.HCl$ (*N,N*-Dimethyl imido dicarboximide diamide). Metformin is unmetabolized and widely distributed to all body tissues including the intestine, liver and kidney. Metformin is also excreted unchanged [1, 2]. Metformin undergoes a methylation process binds to the monoamine transporter called organic cation transporter group (SLC 29A4/SLC 22A1/SLC 47A1), then plays a role in the redox reaction of the DNA synthesis process and stimulates AMPK through inhibition of the mitochondrial complex I reaction and activation of mitochondrial reactive nitrogen species (RNS) and phosphoinoside-3-kinase (PI3K) [2].

Metformin use oral anti-diabetic in type-2 DM patients for almost a decade [3], works through AMPK activation, thus increase insulin receptor sensitivity. AMPK activation also inhibits hepatic gluconeogenesis and glycogenolysis process and then glucose uptake may increase. This process may lead the increase of lactic acid production, especially when anaerobic glucose metabolism occurs. In addition, metformin effectively increase insulin activity in the musculoskeletal and liver by doing exercising or active physical activity thereby increasing energy requirements and metabolic responses throughout the body (**Figure 1**).

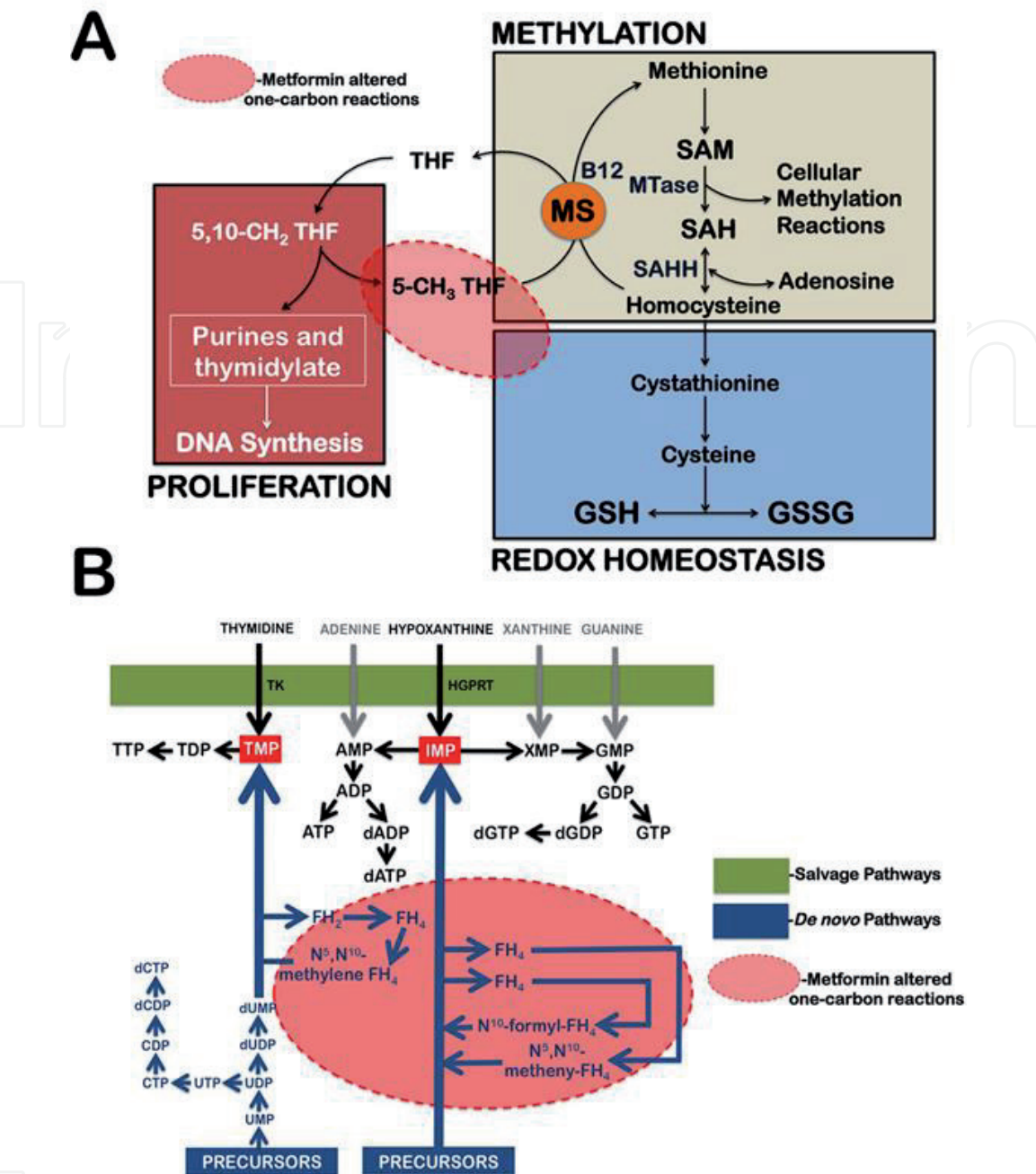


Figure 1.
Methylation process of Metformin in Krebs' cycle [4].

Since, metformin inhibits hepatic gluconeogenesis process, it also reduces metabolic acids flux thus lactic acid accumulated and may lead to metformin associated lactic acidosis (MALA) [5] as seen in **Figure 2**. However, MALA is rarely happened [3, 7].

Indications of metformin treatment in Type 2 DM patients is HbA1C levels within range of 7–8%. Moreover, metformin also use to improve insulin receptor resistance through the AMPK pathway in pre-diabetes type 2 patients with impaired glucose tolerance, obese patients and polycystic ovaries. Contra indication of metformin use: pregnancy and breastfeed, renal insufficiency, liver failure, heart failure lactic acidosis, severe infection, dehydration and alcoholism.

Metformin is excreted by the kidneys in an unchanged form, thus patient with renal dysfunction need to be carefully given [8]. Some studies suggested metformin may still be given to patients with impaired renal function and does not require a dose adjustment whenever the GFR is >40% [9, 10].

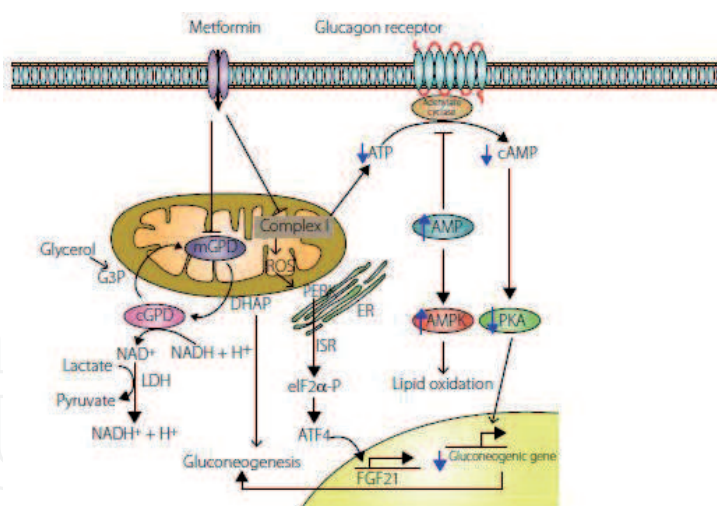


Figure 2.
 AMPK stimulation pathway by metformin [6].

2. Tuberculosis, its therapy and resistance mechanism

Tuberculosis (TB) is a chronic infection caused by *Mycobacterium tuberculosis* (Mtb). Tuberculosis remains one of “global health emergency” diseases [11]. Nowadays, the evidence of TB new cases are increasing and some studies said that this situation were associated with the rising number of patients with immunocompromised condition such patients with HIV, diabetes mellitus (DM), cancer and autoimmune diseases [12, 13]. The risk of TB infection in DM patients increased 2,39 times. Moreover, the risk of failure of anti-TB therapy in DM patients also increased 1.69 times [14, 15].

Tuberculosis continues to be difficult to treat, mainly due to three natural barriers: 1) **Cell wall.** *M. tuberculosis* (M.tb) has a waxy appearance, which is due to the composition of the cell walls. More than 60% of the cell wall is lipid, mainly mycolic acids. This extraordinary membrane of M.tb obviates many pharmacological compounds from penetrating the cell membrane or getting inside the cytosol; 2) **Efflux pumps.** The second layer of M.tb defense is provided by the ability of efflux pumps in the cell membrane. Potentially harmful chemicals are pumped out from the bacterial cytoplasm into the extracellular space by these transport proteins. This process contributes to the resistance of mycobacteria to anti-tuberculosis standard; 3) **Location in host.** A third barrier is the propensity of some of the bacilli to hide inside the patient’s cells, thereby surrounding themselves with an extra physico-chemical barrier that antimicrobial agents must cross to be effective [2, 16].

First-line anti-TB regiments are rifampicin, isoniazid (INH), ethambutol, pyrazinamide, use as anti-TB. Second line of anti-TB are fluoroquinolone (ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin), ethionamide, PAS, cyclomerize, amikacin, kanamycin, dan capreomycin [2]. Herewith, we discussed more in First-line anti-TB.

Rifampicin are macrocyclic antibiotics. Rifampin or rifampicin, rifapentine, and rifabutin are macrocyclic antibiotics important in the treatment of mycobacterial diseases. Rifampicin binds to the β subunit of DNA-dependent RNA polymerase (*rpoB*) to form a stable drug-enzyme complex, then suppresses chain formation in RNA synthesis [2]. Rifampicin should be taken on an empty stomach, whereas rifapentine should be taken with food if possible. Rifampicin is mostly well tolerated in patients. Less than 4% of patients with TB developing significant adverse reactions; the most common are rash (0.8%), fever (0.5%), and nausea and vomiting (1.5%). Rifampicin is a hepatotoxic agent, however, rarely, hepatitis and deaths

due to liver failure in whom had pre-existing liver disease. Chronic liver disease, alcoholism, and old age appear to increase the incidence of severe hepatic problems. GI disturbances have occasionally required discontinuation of the drug. Rifampicin potently induces CYPs 1A2, 2C9, 2C19, and 3A4, and it decreases the $t_{1/2}$ of zidovudine, prednisone, digitoxin, quinidine, ketoconazole, propranolol, phenytoin, sulfonyleureas, hormonal contraceptive and warfarin [1, 2].

Isoniazid (*isonicotinic acid hydrazide*), also called INH is an important drug for the chemotherapy of drug-susceptible TB. INH enters M.tb membrane cell by passive diffusion. INH is not directly toxic to the bacillus, it must be activated into toxic form for M.tb by KatG, a multifunctional catalase-peroxidase. INH active metabolite, isonicotinoyl, was the product of KatG catalysation. Isonicotinoyl interacts with M.tb's NAD and NADP then produce toxin. The nicotinoyl-NAD isomer, inhibits the activities of enoyl acyl carrier protein reductase (InhA) and KasA. Inhibition of InhA and KasA inhibits synthesis of mycolic acid of the mycobacterial cell wall and then leads to bacterial cell death. A nicotinoyl-NADP isomer, another toxic product of isonicotinoyl-NAD and NADP interaction, inhibits ($K_i < 1$ nM) M.tb dihydrofolate reductase, then interferes nucleic acid synthesis. KatG activation of INH also produce superoxide, H_2O_2 , alkyl hydroperoxides, and the NO radical. These products may also contribute to the INH's mycobactericidal effects, due to the defect of M.tb in the central regulator of the oxidative stress response, *oxyR*. Backup defense against radicals is provided by alkyl hydroperoxide reductase (encoded by *ahpC*), which detoxifies organic peroxides. Increased expression of *ahpC* reduces isoniazid effectiveness [2].

Isoniazid is metabolized by hepatic arylamine NAT2. The patients' clearance of INH classifies into three phenotypic groups: fast, intermediate, and slow acetylators. These acetylator groups relates to NAT2 genotype and influenced by race, not by sex or age. Fast acetylation is found in Inuit and Japanese, while slow acetylation is the predominant phenotype in most Scandinavians, Jews, and North African whites [2]. The high acetyltransferase activity (fast acetylation) relates to high dose demand of INH. After NAT2 converts isoniazid to acetyl isoniazid, which is excreted by the kidney, acetyl isoniazid can also be converted to acetyl hydrazine and then to hepatotoxic metabolites by CYP2E1. Drug-Induced Hepatitis (DIH) associated INH occurs ~0.1% of all patients taking INH. Hepatic damage incidence increases with age but is rare in patients less than 20 years old. The risk is increased ~3% by coadministration INH with rifampicin. Most cases of DIH occur 4–8 weeks after initiation of anti TB therapy [2, 17]. Neuropathy, such as peripheral neuritis (most commonly paraesthesia of feet and hands) is more frequent in slow acetylators and in individuals with diabetes mellitus, poor nutrition, or anemia. To prevent neuropathy, pyridoxine is needed. Isoniazid may also induce syndrome resembling systemic lupus erythematosus. Isoniazid is a potent inhibitor of CYP2C19 and CYP3A and a weak inhibitor of CYP2D6. However, isoniazid induces CYP2E1. Herewith drugs that are metabolized by these enzymes will potentially be affected (**Table 1**) [1, 2].

Pyrazinamide is the synthetic pyrazine analogue of nicotinamide and activated by acidic conditions. Pyrazinamide as anti TB has several mechanisms of action. Pyrazinamide passively diffuses into M.tb cells, and then pyrazinamidase (encoded by the *pncA* gene) deaminates pyrazinamide to pyrazinoic acid (POA–, in its dissociated form). Pyrazinoic acid passively diffuses to POA– to the extracellular milieu. In an acidic extracellular milieu, a fraction of POA– is protonated to the uncharged form, POAH, a more lipid-soluble form. The POAH re-enters back to M.tb cells and accumulates due to a deficient efflux pump [2, 18]. The acidification of the intracellular milieu is believed to inhibit enzyme function and collapse the transmembrane proton motive force, thereby killing the bacteria. Inhibitors of

No	Coadministration Drug	CYP isoform	Adverse Effects
1.	Acetaminophen	CYP2E1 induction	Hepatotoxicity
2.	Carbamazepine	CYP3A inhibition	Neurological toxicity
3.	Diazepam	CYP3A and CYP2C19 inhibition	Sedation and respiratory depression
4.	Ethosuximide	CYP3A inhibition	Psychotic behaviors
5.	Isoflurane and enflurane	CYP2C19 inhibition	Decreased effectiveness of INH
6.	Phenytoin	CYP2C19 inhibition	Neurological toxicity
7.	Theophylline	CYP3A inhibition	Seizures, palpitation, nausea
8.	Vincristine	CYP3A inhibition	Limb weakness and tingling
9.	Warfarin	CYP2C9 inhibition	Bleeding (higher risk with INH doses >300 mg/d)

Table 1.
Drugs interact with Isoniazid [2].

energy metabolism or reduced energy production states lead to enhanced pyrazinamide effect. A specific target of pyrazinamide has been proposed to be ribosomal protein S1 (encoded by *RpsA*) in the trans-translation process, so that toxic proteins due to stress accumulate and kill the bacteria. In addition, pyrazinamide's target may include an aspartate decarboxylase (encoded by *panD*) involved in making precursors needed for pantothenate and CoA biosynthesis in persistent Mtb Injury to the liver is the most serious side effect of pyrazinamide. Therefore, all patients should undergo examination of hepatic function prior to pyrazinamide administration to prevent drug-induced hepatitis, and should be repeated at frequent intervals during the entire period of treatment. If evidence of significant hepatic damage becomes apparent, therapy must be stopped. In an individual with hepatic dysfunction, pyrazinamide should not be given unless this is absolutely unavoidable. Pyrazinamide inhibits excretion of urate in nearly all patients, which may cause acute episodes of gout due to hyperuricemia condition. Other untoward effects observed with pyrazinamide include arthralgias, anorexia, nausea and vomiting, dysuria, malaise, and fever. Because of insufficient data on teratogenicity, the use of pyrazinamide is not approved during pregnancy in the U.S. [1, 2].

Ethambutol hydrochloride is a water-soluble and heat-stable compound. Ethambutol inhibits arabinosyl transferase III, thereby disrupting the transfer of arabinose into arabinogalactan biosynthesis, which in turn disrupts the assembly of mycobacterial cell wall. The arabinosyl transferases are encoded by *embAB* genes. The oral bioavailability of ethambutol is about 80%. Approximately 10–40% of the drug is bound to plasma protein. The decline in ethambutol is biexponential, with a *t*_{1/2} of 3 h in the first 12 h and a *t*_{1/2} of 9 h between 12 and 24 h due to redistribution of drug. Clearance and Vd are greater in children than in adults on a per kilogram basis. Slow and incomplete absorption is common in children, so that good peak concentrations of drug are often not achieved with standard dosing. About 80% of the drug is not metabolized at all and is renally excreted. Therefore, in renal failure even in patients receiving hemodialysis, ethambutol does not need dose adjustment. Ethambutol induces very few serious unfavorable reactions: About 1% experience diminished visual acuity, 0.5% a rash, and 0.3% drug fever. Other side effects that have been observed are pruritus, joint pain, malaise, GI upset, abdominal pain, dizziness, headache, disorientation, mental confusion, and possible hallucinations. The most important side effect is optic neuritis, resulting in decreased visual acuity and loss of red-green discrimination. Therefore, ethambutol should not be given in children and pregnancy.

The aim of combination anti TB are 1) increasing bactericidal activity from the very beginning of therapy and 2) preventing pathogen resistance, therefore, patients could be cured. Prevent to death, prevent to recurrence, and cutting off the transmission chains by eradicated Mtb [8, 19]. Rifampicin and isoniazid have the highest bactericidal activity against Mtb, compared to other anti-TB. However, rifampicin and isoniazid are also easily becoming resistance.

Herewith some mechanism of anti-TB resistance: 1) Anti-TB amenable to penetrate into Mtb wall's cell, due to its rich of lipopolysaccharide and mannose; 2) Mtb becomes dormant easily in anaerobic, thus anti-TB, except rifampicin and fluoroquinolone, which aims to inhibit metabolic processes became ineffective in dormant conditions; 3) Alteration of the enzymes that responsible for activating pro-drugs (pyrazinamide and isoniazid); 4) DNA of Mtb mutates; 5) Target protein's structure alters thus the efficacy of rifampicin, ethambutol, streptomycin, fluoroquinolone and macrolide declined (**Figure 3**).

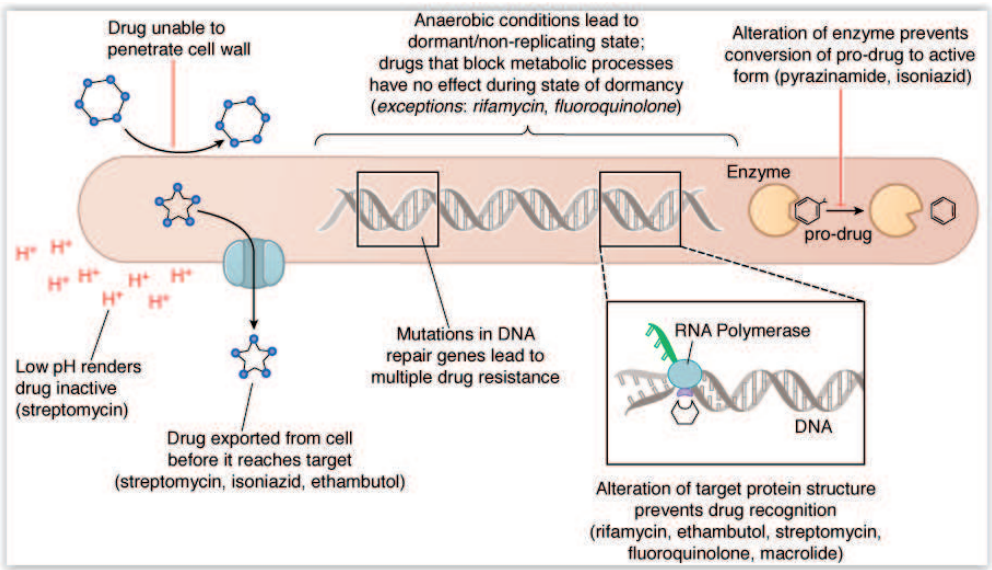


Figure 3.
Anti-TB resistance mechanism against Mtb [2].

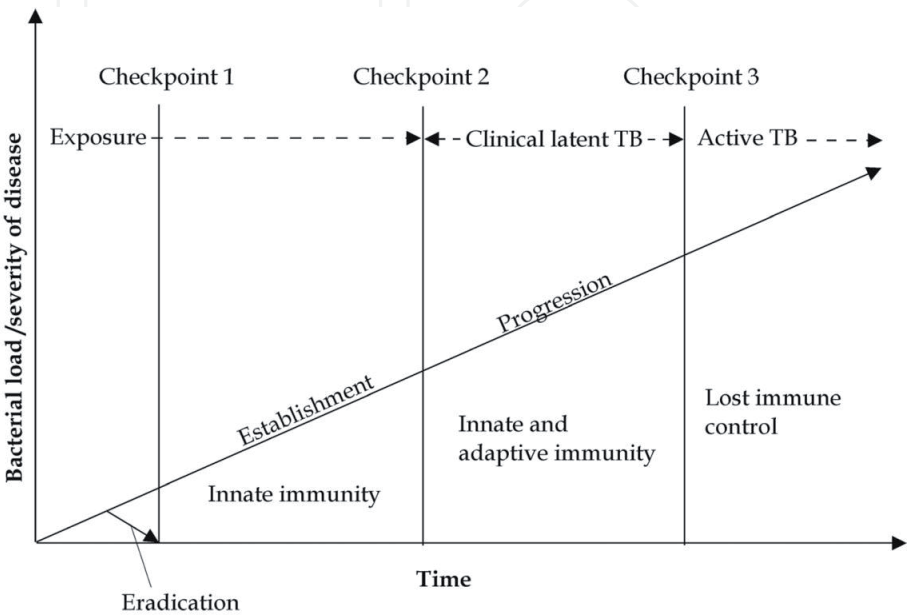


Figure 4.
Escape mechanism of Mtb [20].

Based on in vitro studies, Rifampicin inhibits Mtb at a concentration of 0.06–0.25 mg/L. The prevalence of rifampicin resistance isolates (1 in every 10^7 to 10^8 CFU bacilli). Pyrazinamide has antimicrobial activity in vitro at an acidic pH of 5.8–5.95 and 80–90% of clinical isolates have an MIC (minimum inhibitory concentration) of 100 mg/L. Pyrazinamide resistance occurs due to single point mutations (pncA gene). The minimum inhibitory concentration (MIC) of isoniazid is 0.025–0.05 mg/L. The prevalence of isoniazid resistance occurs at 1 in every 10^6 CFU bacilli. The inhibition of ethambutol is 0.5–2 mg/L and resistance occurs due to the embB gene mutase. Based on this, to prevent anti-TB resistance, it is given in combination [2].

Despite of those anti-TB resistance, Mtb also has ability to manipulate host immune response, both innate and adaptive immune systems or known with escape mechanism (**Figure 4**). Mtb has ability to avoid intracellular killing inside macrophages (phagocytosis) [20].

3. Immune response against *Mycobacterium tuberculosis*

Several epidemiological models of family members who have long shared the bedroom with subjects with TB infection have clearly demonstrated that 5 to 20% of them do not get infected (resilient individuals or resisters), or become transiently infected (early sterilization or early clearance) [21]. An individual defined as resilient after close and prolonged contact with the negativity both of the skin reactivity test and of the IFN- γ release assay (IGRA) which persists for at least 1 year. On the other side, the study of TB susceptibility, has reported onto various components of human immunity to mycobacteria. Different genetic polymorphisms which modulate the host immune response in favor of TB infection and disease progression have been identified in human leukocyte antigens (HLA), toll like receptors (TLR), vitamin D receptors (VDR), cytokines with their receptors and many other functional immune components [21].

Transcriptomic studies have described a TB signature of neutrophil-driven IFN-inducible genes in Mtb, including IFN- γ but also type I IFNs, reflecting disease extension and response to treatment and highlighting the previously under-appreciated role of IFN- α signaling in TB pathogenesis. Beyond host factors, bacterial virulence constitutes the other major player when evaluating the risk of TB infection. Differential Mtb gene expression in the different phases of infection also contributes to the bacterial virulence besides bacterial strain or burden in respiratory secretion. Mtb lacks virulence factors such as toxins, and its immune-escaping ability depends on the alteration of lipid metabolism, metal-transporter proteins, and protease, which inhibit the antimicrobial effectors of macrophages [22].

Macrophages are the first line of immune defense, so they can prevent the infection but only if the ratio of forces lies clearly to their advantage [23]. Otherwise, they favor its development because they become first a niche for the slow replication of the Mtb and then the sanctuary for the persistence of the infection inside the phagosome during the latent infection phase. Mtb expresses an extremely wide variety of virulence factors that counteract macrophage ability in suppressing the pathogen. Among Mtb strategies we can include the intracellular trafficking inhibition, autophagy inhibition, cytosol entry ability, the induction of host cell death and the neutralization of toxic components as reactive oxygen species [15, 21].

Whilst IFN- γ is a key element in the containment of Mtb within the Macrophage, it is now widely recognized that performing this function requires the presence of vitamin D. IFN- γ axis is struggling against the ESX-system to enhance

phagolysosome activity, vitamin D deficiency abets the Mtb replication [21]. IFN- γ is the chief cytokine involved in the protective immune response against mycobacterial infection [24–26]. The main function of IFN- γ is macrophage activation, thus in this study autophagy marker was also high [27], it referred to its mycobactericidal functions. Predominantly IFN- γ is also contributed to less severe forms of pulmonary TB [28]. Moreover, IFN- γ also enhances the antigen presentation through the induction of the expression of molecules from the major histocompatibility complex (MHC) class I and II and promoting the differentiation of CD4 T lymphocytes to the Th1 subpopulation [26, 29]. Furthermore as conclusion, MET through mTOR inhibition enhances macrophage's autophagy activity thus Th1-related IFN- γ activity increases and in this study, DM-TB coinfection patients represented by BTA conversion. However, IFN- γ relates to CD8 T lymphocytes or cytotoxic T-cells activity which contributes to lung tissue damaged, thus IFN- γ activity needs to be controlled [28, 29].

IL-10 is produced by macrophages and Th-2 during *M. tuberculosis* infection. IL-10, through SOCS 3 activation, acts inhibiting target cells of inflammation, then the production of pro-inflammatory cytokines (IFN- γ , TNF- α and IL-12) reduced [24, 25, 28]. Due to its ability to inhibit the production of pro-inflammatory cytokines, IL-10 has an immune-regulatory function which plays an important role in adequate balancing between inflammatory and immune-pathological responses. However, the increase in IL-10 levels appears to support the mycobacterial survival in the host [28]. IL-10 reduces the protective response to *M. tuberculosis* by inhibiting autophagy targeting signals through IL-10 activated SOCS3, and then, SOCS3 inhibits the Janus kinase-2 (Jak2)/signal transducer and activator of transcription (Stat) pathway in activating macrophage autophagy [24, 25]. In this study, the increasing of IL-10 not only due to macrophage related Th-2 activation, it was insulin attenuated anti-inflammation regulatory. In this study, insulin was used for patients 'hyperglycemic condition [30, 31].

Nitric oxide (NO) within macrophages play less important role in human. On the other hand, reactive oxygen species (ROS) play a well-documented role in the immune response to Mtb, which increases susceptibility in patients displaying mutations in a catalytic subunit of NADPH-oxidase 2 involved in ROS production on phagolysosome membrane. Mtb affects NADPH-oxidase activity through nucleoside diphosphate kinase (Npk) interaction with small GTPases involved in NADPH-oxidase assembly and functioning [21, 32].

Dendritic cells (DCs) play a fundamental role in the immune defense system due to antigen presentation, co-stimulating activity and the large cytokine production capacity with activity on the lymphocytes cluster of differentiation (CD) 4. DCs role in immune response against TB remains controversial. DCs soon become a niche for the Mtb. CD209, also called DC-specific intercellular adhesion molecule 3-grabbing non-integrin receptor (DC-SIGN), represents the gateway of Mtb into the DC [33].

The T lymphocytes immune response begins when Mtb spreads inside the lymph nodes, but its arousal lies in the early activation of the innate immune system. Inside the lymph nodes, T lymphocytes undergo a process of activation and expansion of the specific populations for the Mtb antigens. However, at this point, the largest part is done and the infection is now established. The development of a hypersensitivity response (delayed-type) to intradermal injected tuberculin (DHT) or purified protein derivative indicates cellular immune response in 2–6 weeks after Mtb infection. It is important to underline that DHT positivity does not correlate with protective response to TB, and the disease can occur in people with adequate DHT response [34, 35].

The process of maturation of the phagosome of macrophages is facilitated and increased by IFN- γ , the production of which is mostly dependent on the T lymphocytes CD4⁺ with a minor support of lymphocytes CD8⁺ and T lymphocytes with $\gamma\delta$ receptor. However, IFN- γ is inadequate to control the infection alone, and it requires the association of other molecules such as IL-6, IL-1, and TNF- α . It is known that TNF- α boosts the production of NO by macrophages and stimulates the production of the chemokines CCL5, CCL9, CXCL10, and CCL2, which then attract immunity cells at the site of infection [21, 36].

T lymphocytes CD8⁺ had no role in controlling the infection and Mtb disease. An activity against Mtb is conceivable considering that T lymphocytes CD8⁺ recognize Mtb antigens through class I molecules of the major histocompatibility complex (MHC), and produce IL-2, IFN- γ and TNF- α , which have a well-known role in controlling Mtb. This direct cell-to-cell contact determines the apoptosis of the Mtb-infected cell (especially macrophages) depriving Mtb from its natural growth environment and at the same time reducing its viability by unknown mechanism [37]. On the other hand, lymphocytes CD8⁺ produce IL-10 and TGF- β which instead favor the development of the Mtb infection.

4. Host-directed therapy for tuberculosis

The effectiveness of anti-TB are also influenced by the host immune response due to the interaction of anti-TB. Immuno-modulators' adjunctive therapy that enhance TB might be able to shorten treatment durations and improve TB outcomes [38, 39]. To identify new host-directed therapy (HDT) for TB patients is WHO's priority for TB management. Nowadays, Host-directed therapy (HDT) provides a largely unexploited approach as adjunctive anti-TB therapy. Firstly, HDT may impair Mtb replication and survival by disrupting Mtb manipulation of macrophage pathways, thus rendering the bacteria more sensitive to host defenses. The current search for novel therapeutics has focused on the use of repurposed drugs aimed at optimizing the host's response against the mycobacterium [40]. HDT has been proposed as adjuvant therapy for TB infection to improve the efficacy of current treatment outcomes. One possible solution to antibiotic resistance and non-replicating bacterial death problem is targeting the host instead of the pathogen.

Several studies about corticosteroids, TNF blockers, thalidomide, and non-steroidal anti-inflammatory (NSAIDs) have been conducted to determine the function of these immunomodulatory agents as an adjunct of OAT therapy [39, 41]. One of HDT mechanism is autophagy due to its ability in inhibiting the TB infection process [39, 41]. The process of activating autophagy from formation to maturation and then fusion with lysosomes for phagolysosome or autophagy processes requires many activators and protein (ATGs), one of the proteins representing phagolysosome or autophagy is MAP1LC3B/ATG8.

5. Mechanism of action of Metformin as candidate for host-directed therapy in patients with diabetes mellitus: tuberculosis coinfection

Metformin (MET) is a Food and Drug Administration (FDA)-approved drug, biguanide the oral anti diabetic agent, well-known for its glucose-lowering effect on type 2 diabetes (T2D) individuals [1, 2]. A group of studies have reported the potential role of MET as an adjunctive therapy for TB [32, 42, 43]. However, the exact mechanism of how MET modulates the cellular interaction between Mtb and

macrophages is not well known. Therefore, we pursued to amalgamate the evidence base on MET as an adjunctive therapy for TB infection using a scoping study methodology to identify gaps to be attained in future research.

Metformin (MET) is the most commonly prescribed drug for type 2 diabetes mellitus. MET through in silico studies, in vitro studies and in vivo studies using animal models, expressed as important role for anti-tuberculosis through immunomodulatory mechanism [42–44], as it is seen in **Figure 5**.

Metformin hydrochloride (MET), recently known has possibilities of utilizing as a combination drug with existing antibiotics for TB therapy [15, 44] and by an extensive in vitro study, MET was reported controlling the growth of drug-resistant Mtb strains via production of mitochondrial reactive oxygen species and facilitates phagosome-lysosome fusion [3, 42]. Thus, MET is known as one of highly potential HDT due to target autophagy by AMPK activation or known as mTOR inhibitor [42, 43].

Moreover, MET is not metabolized by P450 enzymes [1, 2, 45], thus it has no interaction with rifampicin that could decrease the therapy efficacy. However, interaction MET and Rifampicin increases the expression of organic cation transporter (OCT1) and hepatic uptake of metformin, leading to an enhanced glucose lowering [46, 47]. MET is also expected enhanced Isoniazid (INH) efficacy due to SOD activity [48]. INH a pro-drug, its activation is requiring an interaction with Kat-G produced by *Mtb* [1, 2]. Kat-G activation also produces oxidative stress – reactive oxygen species (ROS), namely H₂O₂ and alkyl hydro-peroxides. ROS is neutralized by an antioxidant, superoxide dismutase (SOD). It assumed that SOD contributes to the INH-induced bactericidal effects [32].

5.1 Metformin dan superoxide dismutase (SOD)

Superoxide Dismutase (SOD) is an enzyme produced by the host antioxidant defense system. Increased reactive oxygen species (ROS) as respiratory burst in TB infection results in macrophage phagocytosis process against Mtb. Massive production of ROS also associated with Th1 overactivation, macrophage activity inhibition, and tissue damage. Hyperglycaemia condition could increase ROS production, therefore SOD levels could also increase in DM patients [49]. KatG gene activates INH from pro-drug to active drug. Apparently, SOD contributes during

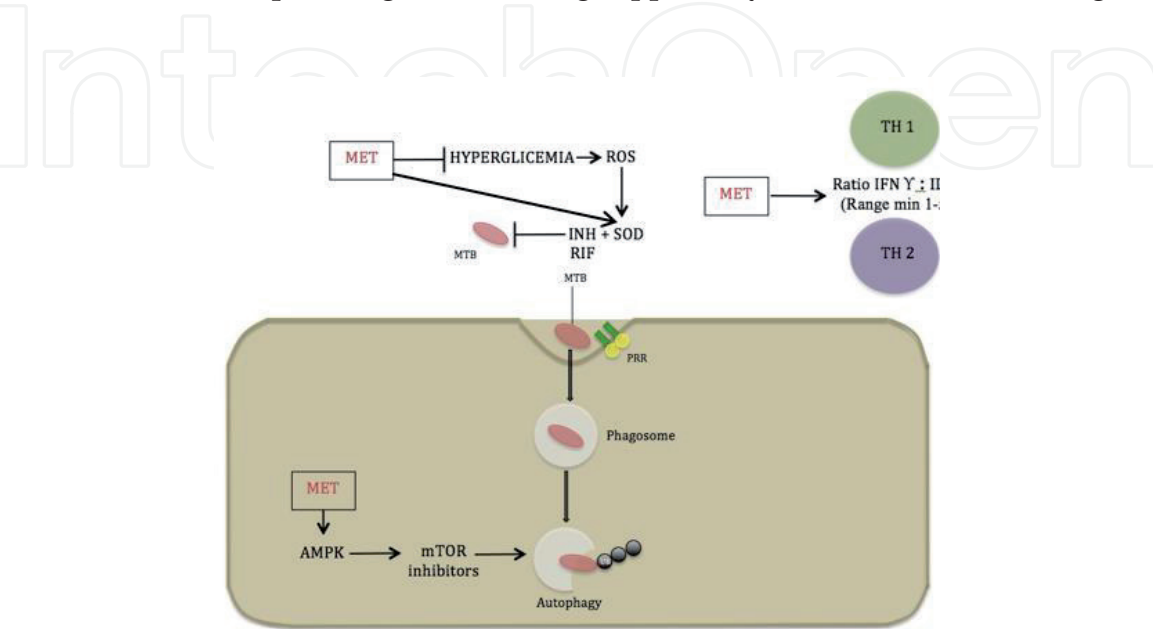


Figure 5.
Mechanism of action Metformin as adjuvant therapy for DM-TB coinfection [32].

this mechanism, higher SOD related to better of INH's in inhibiting Mtb [48]. MET has ability in improving SOD level during inflammation [50, 51]. Based on this, the addition of MET provides synergism effects to increase the effectiveness of INH in treating TB infection. MET also has a synergistic effect with RIF through increasing the expression of organic cation transporter (OCT)-1. The OCT-1 expression plays a role in inhibiting transcription Mtb [9, 44]. Moreover, target of glycemic level for DM-TB patients is also need to be adjusted, therefore synchronized with SOD production [15].

5.2 Metformin induced autophagy

Mtb has an escape mechanism through inhibition of host macrophage cells' autophagy [20, 38]. Improving the autophagia process will improve anti-TB in eliminating Mtb. MET activates Adeno Monophosphate Kinase (AMPK) and subsequent phosphorylation of unc-51-like kinase 1 (ULK1) [52], then AMPK works as mTOR inhibitor and enhances autophagy [37, 39, 42, 43]. Therefore, MET from pharmacodynamics aspect has no effect to Mtb but works on host immune regulation [6, 15].

5.3 Metformin, *interferon gamma* (IFN- γ), *interleukin* (IL)-10 and its ratio

In chronic TB infection, IFN- γ level increases as the body cellular immune response. Currently, IFN-release assay (IGRA) is used as a diagnostic tool for latent TB infection and as an indicator of therapeutic success in active TB infection [26, 53, 54]. IL-10 is a negative feedback regulator on the immune response produced by Th2 to inhibit excessive production of pro-inflammatory cytokines. IL-10 barriers the macrophage function, due to suppression of MHC class II molecules and reduces co-stimulator expression [55–57].

MET associated AMPK activation, through thioredoxin-interacting protein (TXNIP) decreases activation of inflammatory mediators and transcription factors, including NF kappa B which encodes proinflammatory mediators [58, 59]. In addition, in intracellular infections such TB MET through AMPK is also stimulated macrophage autophagy [15, 52], therefore MET accelerates Mtb elimination process without excessive inflammatory processes that can damage the tissue [22].

6. Side effects of Metformin that might occur

Gastrointestinal disorders (anorexia, nausea, vomiting and diarrhea) is one of the most common MET's side effect. Impaired absorption of vitamin B₁₂, impaired liver and or kidney function or in elderly people [1, 2]. Increased levels of lactate or known as Metformin-associated lactoacidosis (MALA) although the occurrence is low, must still be prevented. MALA is a life-threatening event. However, in Diabetes Tuberculosis coinfection patients, MALA could be prevented by determining patients criterias, including: 1) has minimal - moderate advaced pulmonary lesions in X-ray chest examination; 2) has oxygen saturation has at least above 92%; 3) has normal liver function (SGOT, SGPT) and normal kidney function (BUN, SK). Providing consultation, information and education related to the symptoms of lacto-acidosis is also needed during MET additional therapy. MET may increase lactate but rarely increase the risk of DM-TB coinfection patients experience MALA [7]. Therefore, MET is relatively safe to use for DM-TB coinfection patients [32].

Abbreviations

TB	Tuberculosis
MET	Metformin
Mtb	<i>Mycobacterium tuberculosis</i>
HDT	Host-directed therapy
INH	Isoniazid
DCs	Dendritic cells
IL	Interleukin
INF	Interferon

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