Review

From natural medicines to metal weapons: therapeutic potential of flavonoid metabolites in regulating host-Mycobacterium tuberculosis metal interactions

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Abstract

Tuberculosis, an infectious disease, causes considerable morbidity and mortality. Within the host immune system, transition metals play vital roles in combating Mycobacterium tuberculosis (MTB), acting as catalytic cofactors, mediating electron transport, and catalyzing antimicrobial radical formation. Both the host and MTB have developed mechanisms to maintain metal homeostasis. Flavonoids, important herbal materials with potent iron-chelating ability, have gained attention for their antitubercular and anti-inflammatory effects, making them a promising treatment option. This review explores how trace metals restrict MTB and the dynamic balance between pathogen and host, revealing innovative perspectives for therapeutic interventions.

Keywords: Mycobacterium tuberculosis; flavonoid; metal ions homeostasis; zinc oxide nanoparticles

1 Introduction

Tuberculosis (TB) is a severe and protracted disease caused by the pathogen Mycobacterium tuberculosis (MTB), and it is the second most significant infectious threat following COVID-19. The emergence of multi-drug-resistant (MDR) strains of MTB makes it necessary to research prevention and treatment strategies immediately [1].

Metal ions, such as zinc, iron, and copper, are indispensable nutrient metals in organisms that implement their catalytic features as enzymatic cofactors, immunological reactions, physiological mechanisms including electron transport, and the stabilization of protein structure [2]. These metals participate in maintaining cell survival, growth, and replication [3]. Furthermore, they are applied to proliferate and resist the antimicrobial radicals of bacterial pathogens [4].

Due to the excess or lack of trace metals in the intracellular microenvironment, MTB may be poisoned or nutritionally deficient [5]. The innate immune system sequesters Fe^{3+} and Zn^{2+} to deprive bacteria of essential nutrients, releases Cu^{2+} to intoxicate them, or

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elevates Zn^{2+} level to suppress pathogen growth [6]. These metals are related to metalloproteins, metalloenzymes, and transporter proteins, and participate in cellular processes such as transcription and signal transduction [7]. The change of intracellular metal concentration can disrupt homeostasis. For instance, excessive metal can generate reactive oxygen species (ROS), inducing toxic effects through the Fenton reaction [7]. Host organisms have evolved mechanisms to modulate physiological metal concentrations through uptake, storage, sequestration, secretion, and scavenging processes. These efforts to starve pathogens from nutrient metals are addressed as nutritional immunity [8]. Meanwhile, MTB regulates the transport of metals through the process involving regulatory proteins that act synergistically to modulate the expression of metal ion transporter proteins, maintaining metal ion homeostasis and resistance necessary for survival within host organisms [9].

Zinc oxide nanoparticles (ZnO NPs) are expected to be used in antibacterial applications because of their ability to generate ROS and release Zn^{2+} [10]. ZnO NPs exhibit a synergistic bactericidal effect when combined with other elements or antibiotics [11-14]. Additionally, green synthesis method using plant extracts is attracting attention as an eco-friendly approach to potentially enhance antimicrobial efficacy [15,16]. People are exploring them as effective strategies to treat TB and MDR TB.

Flavonoids are important active substances in traditional Chinese medicine and have strong iron chelating activity in 6,7-dihydroxy [17]. Many flavonoid drugs have demonstrated anti-tuberculosis activity [18]. Baicalin, as an active component in the traditional Chinese medicine *Scutellaria baicalensis*, has iron-chelating properties. Its most effective iron-binding site arises from the C6, C7-dihydroxyl groups and a C2, C3-double bond in its structure can inhibit the formation of MTB biofilms and reduce the inflammatory response of the body at the same time [19]. Although the relationship between the anti-tuberculosis effect of flavonoids and metal ion chelation needs to be further clarified, it provides a new idea for the treatment of tuberculosis.

As a result, elucidating the regulatory mechanisms of metal maintenance is helpful to decipher the function of trace metal ions in host-MTB interaction, and provide innovative therapeutic strategies for coping with the emergence of MDR strains [20]. In this review, we focus on comprehensively understanding the interaction mechanism of regulating metal ion homeostasis between MTB and host cells to provide insights for feasible immunotherapeutic strategies.

2 Zinc homeostasis

2.1 Physiological effects of zinc

Zinc is a decisive trace metal that accounts for approximately 10% of the mammalian genome, encoding zinc-binding proteins that serve as cofactors for enzymes [21]. Zinc is involved in enzymatic reactions such as transfer reactions, and also contributes to DNA binding protein function [20]. The change of zinc concentration enhances immune functions including macrophages' antibacterial capacities and promotes dendritic cell maturation [22,23]. Zinc has higher affinity for binding non-homologous proteins in MTB than other metals at high Zn^{2+} level. These actions maximize protein dysfunction, which constrains the survival of MTB [24].

Zinc exhibits potent antioxidant properties in the formation of superoxide dismutase, thereby catalyzing the conversion of superoxide radicals into hydrogen peroxide. Therefore, zinc has the potential to alleviate oxidative stress by precluding the Fenton reaction. Zinc deficiency impairs the immune system, resulting in physiological changes such as differential expression of antigens, cytokines, and

major histocompatibility complex class (MHC) on the surface of dendritic cell [25]. Consequently, zinc malnutrition induces the host susceptible to pathogens. However, excessive zinc can destroy immune cells. The accumulation of zinc takes part in the production of ROS, which causes oxidative stress. Moreover, excessive ROS will damage essential cellular components of pathogens, such as proteins and nucleic acids, thus interfering with bacterial growth [26].

2.2 Zinc regulation within the host

Hosts have evolved multitudinous mechanisms to regulate zinc homeostasis, including Zn^{2+} transporters, namely ZIP importers and ZnT exporters. These are zinc transporters that modulate the uptake and secretion of free zinc ions. ZIP family consists of 14 genes (SLC39 family from SLC39A1 to SLC39A14), and ZnT family consists of 10 genes (SLC30 family from SLC30A1 to SLC30A10) [27]. Zinc is transported into innate immune cells by members of the ZIP family transporters [20]. ZIP family mediates zinc import into the cytoplasm, while ZnT1 mediates zinc export into the phagolysosome during infection. Excessive Zn^{2+} is sequestered within organelles, including the endoplasmic reticulum, Golgi apparatus, lysosomes, and zincosomes – membrane bound vesicular structures containing high levels of zinc [28]. Furthermore, the metal-response-element-binding transcription factor 1 (MTF-1) can sense free Zn^{2+} and maintain Zn^{2+} level by regulating the transcription of zinc transporters. Stimulated by cytokines and hormones, the dynamic regulation of zinc transporter transcription profiles plays a pivotal role in the mobilization of zinc [29]. Meanwhile, metallothioneins (MTs) tightly control Zn^{2+} homeostasis. MTs are small proteins, containing conserved cysteines, which can form complexes with up to seven zinc ions through their zinc-sulfur clusters, effectively binding and sequester zinc (and copper) to alleviate the oxidative stress [30], accounting for approximately 20% of intracellular zinc sequestration [31]. The regulation of the MTs promoter involves zinc (MTF-1) and redox reactions, especially nuclear factor erythroid 2-related factor 2 (NRF2) [32]. Stimulated by Lipopolysaccharide (LPS), the host upregulates the MTs expression to achieve intracellular zinc homeostasis and modulate cellular zinc distribution through redox reactions.

2.3 Zinc regulation within MTB

Similarly, the optimal level of zinc is crucial for the survival and replication of MTB. Macrophages consistently transport Zn out of the phagosome via Nramp1 and Nramp2, depending on specific pH conditions. In MTB, zinc is regulated by Nramp transporters (termed as MntH). Beyond that, MTB can secrete and import kupyaphores, which are synthesized by a biosynthetic cluster in the early phases of infections to address zinc deficiency. Kupyaphores serve as metal transporters, promoting zinc acquisition and enabling pathogens to survive in high zinc toxicity environments [33].

During infection, excessive ROS production in the host system leads to oxidative stress as a mechanism to restrict MTB. The expression of proteins involved in DNA replication, repair, recombination, and antioxidant enzymes such as KatG and AhpC is upregulated in Zn^{2+} limited MTB [34]. Zinc limitation also leads to physiological responses, including the upregulation of genes involved in iron storage (*bfrB*) and regulation (*furA*) [35].

The presence of excessive zinc imposed by the host enhances the intracellular killing of bacteria within immune cell. Zn^{2+} detoxification machinery deficient strains are susceptible to host immune defenses both *in vitro* and *in vivo*, as demonstrated in mouse models of infection [20]. MTB harbors the zinc uptake repressor Zur (Rv2359) (used to be

termed as FurB) which controls the transcription of genes related to zinc acquisition [36]. The transcription expression level of Zur is modulated by *smtB*, which is encoded by upstream gene and shares a promoter with *zur*. SmtB functions as a repressor that becomes inactive when it binds to Zn^{2+} [37]. At high metal concentration, the binding of SmtB-zinc significantly reduces its DNA binding affinity, thereby promoting RNA polymerase loading and initiation of operon transcription, ultimately leading to the expression of metal efflux proteins [3[8]]. Zur acts as a transcriptional repressor and upregulates gene expression *in vitro* under zinc-limited conditions. Zur modulates the ESX-3 system, contributing to upregulation of mycobactin and zinc transporter proteins, regardless of whether there is deficiency or excess of zinc in MTB [39]. ESX system consists of five independently functioning subsystems (from ESX-1 to ESX-5) that modulate the host immune defense, and secrete extracellular proteins to maintain survival and virulence for MTB. Among them, ESX-3 system is significant in the growth of pathogen. Not only Zur, IdeR (iron regulator) and MntR (manganese regulator) can also regulate the expression of esx-3 operon and sustain the homeostasis of these metals [40]. ESX-3 secretes a heterodimer consisting of EsxG and EsxH, which is regulated by zinc and iron levels. This impairs the phagosome fusion with lysosomes, and contributes to virulence [40]. The newly identified redox sensor CmtR in Mycobacterium bovis interacts with Zur to inhibit the expression of the *esx*-3 operon in order to regulate the expression of *esx*-3 and accumulate zinc level. CmtR (Rv1994c), a metal-responsive regulatory protein of the ArsR-SmtB family, shows important inducing effect under oxidative stress.

Transcriptional regulators of pathogens sense the intracellular zinc concentration, and MTB employs P-type family ATPases such as CtpC, CtpG and CtpV to actively expel excess zinc, and utilizes zinc-binding chaperone-like proteins such as PacL1 to acquire zinc in a deficient environment to achieve zinc homeostasis [41]. MTB possesses two superoxide dismutases: SodA and SodC. SodA utilizes manganese as its preferred cofactor and depends on CtpC for metalation, and then exports it to the phagosome. Bacterial metallothioneins, such as MymT in MTB and SmtA or SmtB, can bind and sequester zinc within the cytosol. The intramembrane protease Rip1 (Rv2869c) plays a crucial role in MTB replication by detecting and resisting the bactericidal effector of macrophages, although the underlying mechanism is still unclear [42].

3 Iron homeostasis

3.1 Physiological effects of iron

As an essential nutritional metal ion, iron participates in various physiological activities, such as electron transport, peroxide detoxification, and serving as a cofactor for numerous enzymes [43]. Excessive free iron can react with hydrogen peroxide to generate hydroxyl radicals, resulting in oxidative stress that restrains the growth of MTB [44]. The extreme lack of iron in the host leads to the susceptibility to the invasion of MTB.

3.2 Iron regulation within the host

Over 70% of iron in the human body is kinetically trapped in heme, rendering it unable to be absorbed by pathogens [20]. Erythrocyte lysis leads to the release of hemoglobin and heme, which are rapidly bound by haptoglobin and hemopexin. The hemopexin-heme complex is internalized in hepatocytes, macrophages, and neurons. The haptoglobin-hemoglobin complex is taken up *via* CD163 receptor-mediated endocytosis in macrophages [45]. The remaining iron is bound by the plasma transport proteins transferrin and

lactoferrin, and the intracellular ferritin protein is responsible for iron storage to regulate the concentration of free iron in blood [46]. To obtain free iron, macrophages phagocytize and degrade senescent erythrocytes, which are subsequently sequestered within ferritin [47]. Calprotectin is a high-affinity calcium-binding protein that also binds to iron, zinc, and manganese in the bloodstream [48]. The host secretes the siderophore binding protein (NGAL) by neutrophils (also secrete lactoferrin and calprotectin) and epithelial cells, which binds to bacterial siderophores in order to inhibit the iron acquisition by MTB [49].

More and more evidences show the role of hormone hepcidin in host iron homeostasis. Transcriptional activation of hepcidin occurs in response to the increase of iron concentration or the presence of inflammatory cytokines such as IL-6 and IFN-γ. This contributes to the binding and internalization of ferroprotein 1 (FPN-1), an iron exporter that limits plasma iron level and sequesters iron in macrophages. Conversely, when iron level decreases, the body will reduce the expression of hepcidin [50]. FPN-1 exports iron from macrophages to the bloodstream. After that, ferrous iron is immediately oxidized by a specific membrane ferroxidase and loaded as ferric iron onto transferrin for tissue distribution *via* circulation. Transferrin iron is taken up by cells *via* transferrin-receptor mediated endocytosis [51]. To prevent iron overload, macrophages upregulate the expression of hepcidin and FPN-1 to facilitate excessive intracellular iron excretion.

Natural resistance associated macrophage protein 1 (NRAMP1), a macrophage and neutrophil specific antiporter, is primarily located on the late endosomal or lysosomal membranes and related to innate resistance [51]. It facilitates the exchange of bivalent cations, including iron, zinc, magnesium, and manganese, between phagolysosomes and cytoplasm to limit MTB's acquisition of metals [52]. NRAMP1 gene is considered a crucial factor in conferring resistance against MTB. Patients with polymorphism in the NRAMP1's regulatory region are at higher risk of infection, which leads to decrease of expression level [53]. The expression of NRAMP1 also affects other iron-related immune pathways, including the formation of NGAL.

3.3 Iron regulation within MTB

The survival and proliferation of MTB under iron limitation require various strategies to absorb and store iron. The enzyme ferrochelatase (Rv1485) catalyzes the last step of heme biosynthesis, making heme a valuable resource of MTB [54]. Additionally, IsdG family enzymes MhuD and MhuP facilitate heme degradation through an unconventional mechanism to enhance iron uptake and utilization [55]. The inner membrane protein DppABCD is a transporter that introduces exogenous heme from the periplasm to the cytoplasm, rendering this complex indispensable for MTB growth, particularly within macrophages [56]. Recent research indicates that heme synthesized from *de novo* is more bioavailable to MTB compared with heme exogenously scavenged. Furthermore, heme synthesized from *de novo* plays a critical role in protecting MTB from macrophage attacks, and bioavailable heme levels decrease during macrophage assault [57].

MTB secretes two types of siderophores, lipophilic mycobactin (MBT) and hydrophilic carboxymycobactin (cMBT), to bind and acquire non-heme iron [58]. During iron limitation, MTB actively synthesizes deoxy-mycobactin [59]. MBT is located in the membrane, while cMBT, as an iron carrier, can be released externally to obtain insoluble iron from host Fe-binding proteins like transferrin and ferritin [60]. These siderophores demonstrate a superior affinity for ferric binding, exceeding the affinity of transferrin [61], and are sequestered by the IrtAB ATP-binding cassette (ABC) transport system.

Mycobactin is capable of traversing the phagosome membrane in order to scavenge iron from the extracellular transferrin [62]. IrtAB interacts with iron carriers to reduce Fe^{3+} to the more soluble Fe^{2+} form and promote the release of iron. IrtAB transport system enhances the efficient iron uptake from ferric cMBT. Extracellular ferric sources are transported by carboxymycobactin-mycobactin siderophore machinery receptor HupB to MTB envelope [63].

HupB and IdeR are iron-dependent transcription factors, which regulate genes related to iron homeostasis. HupB (Rv2986c) was identified in the cell wall of MTB. HpuB induces the production of siderophores in response to iron starvation and promotes the activation of its own operon in the absence of IdeR-Fe^{2+}. A *hupB* knockout strain exhibits a decrease in siderophores level and *mbt* transcription level, highlighting the ability of HupB to make MTB counteract macrophage defenses [64]. IdeR protein represses the expression of iron uptake genes and biosynthesis siderophores in iron sufficient environments, causing the corresponding activation of iron storage genes. It becomes inactive and expresses the iron uptake genes under iron starvation. IdeR-Fe^{2+} complex binds to the IdeR box in the *mbt* locus and the upstream *bfrA* gene, thus activating the synthesis of iron storage molecules [3,65,66]. The complex can not be formed under the condition of iron restriction, which preventing it from binding to target genes. This allows HupB to bind to the hupB box located upstream of the IdeR box, which is helpful to induce *mbt* genes transcription and promote iron uptake [42,65]. The IdeR-deficient strains are vulnerable in macrophages, highlighting the significance of IdeR in the virulence of MTB [67]. IdeR protein regulates the synthesis of iron storage proteins. Bacterioferritin (BfrA) binds to heme, while ferritin (BfrB) does not bind to heme. The priority of BfrA in iron storage at low iron concentration and the demand for BfrB at high iron concentration are attributed to its higher storage capacity [68], which is three times that of BfrA [69]. The transcription of iron storage genes *bfrA* and *bfrB* is repressed in the absence of the IdeR-Fe^{2+} complex [66]. In addition, IdeR regulates the ESX-3 system, which highly expresses MBT and zinc transporter proteins [39]. Under oxidative stress, Fe^{2+} is oxidized to Fe^{3+}, which leads to the inability of ferrous (Fe^{2+}) iron to bind to IdeR, causing the up-expression of IdeR-suppressed genes such as those in the ESX-3 system [70].

4 Copper homeostasis

4.1 Physiological effects and host regulation of copper

As an antimicrobial agent, high levels of copper accumulate in the phagolysosomes of macrophages to combat infections. Copper can be converted between two oxidation states, Cu^{+} and Cu^{2+}, and has a high redox potential, which enables copper to catalyze the production of hydroxyl radicals under aerobic conditions, causing oxidative stress through Fenton and Haber-Weiss reactions [71]. After stimulated by proinflammatory factors such as LPS and IFN-γ, phagocytic cells upregulate the expression of copper transporter CTR1 and the copper transport ATPase ATP7A. CTR1 promotes free copper to enter cytoplasm. Subsequently, copper chaperone Atox1 captures and transports copper to ATP7A located in the Golgi apparatus. Then ATP7A transports the copper to phagolysosomes. These copper transport mechanisms accumulate copper to toxic levels, produce ROS and lead to respiratory burst, which is an essential immune response during infection [72].

4.2 Copper regulation within MTB

To counteract oxidative stress caused by excess copper in macrophages, MTB has developed mechanisms to regulate copper levels and enhance

resistance to oxidative stress through the action of copper-responsive transcriptional repressors CsoR (Rv0967) and RicR (Rv0190), P-type ATPase CtpV (Rv0969), copper transport protein MctB (Rv1698), metallothionein MymT, and multicopper oxidases MmcO (Rv0846c).

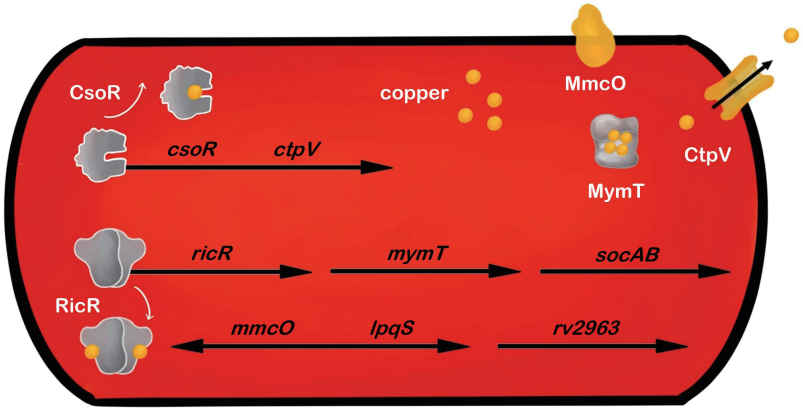


Fig. 1 Copper regulation within MTB

CsoR is a transcriptional repressor that regulates the copper-sensitive operon (*cso*) in a copper-dependent manner, and this operon contains genes for CsoR and CtpV [73]. CsoR binds to DNA without copper, and at elevated Cu^{+} level, it reduces the binding affinity to the promoter of the *cso* operon. This leads to the gradual expression of *cso*, resulting in copper export [74]. Genes involved in the CsoR regulon directly participate in alleviating the detrimental effects of copper stress, rather than CsoR itself [74]. For instance, CsoR regulates the putative copper exporter CtpV. CtpV synthesizes a copper-specific endosomal pump that cooperates with MctB to enhance the copper export across the membrane. CtpV moderates copper homeostasis and alleviates copper oxidative stress. Mice infected with CtpV mutants exhibited slight lung damage and immune response, as well as significantly prolonged survival time compared with those infected with wild-type strains, suggesting the importance of the copper response in MTB. Furthermore, no difference was found between *ctpV*- deficient and wild-type strains in lung bacterial load, suggesting alternative mechanisms may substitute for CtpV's function [75]. The transport of copper involves not only CtpV but also CtpA and CtpB. Although CtpB imports copper to meet nutritional requirements, it can also export excess copper during toxic copper stress periods [76,77].

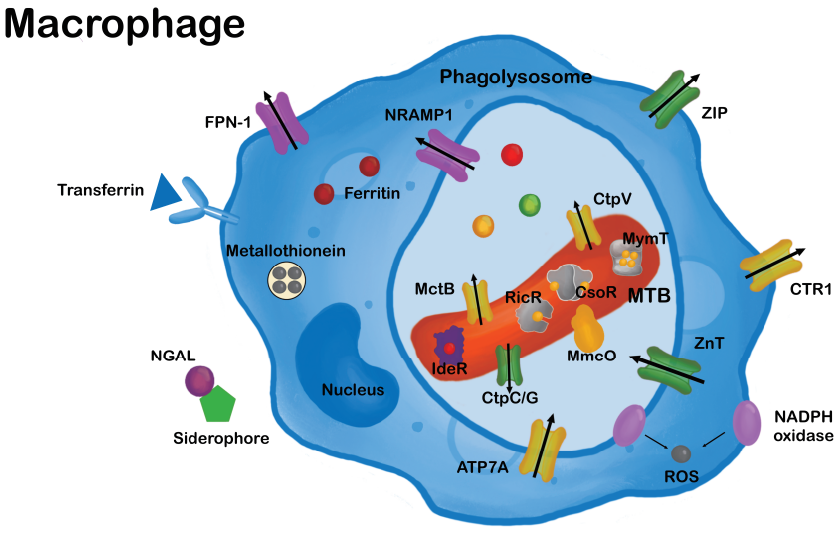
The Mycobacterial copper transport protein B (MctB) is proposed to function as an outer membrane channel, responsible for exporting copper from the periplasmic space to the environment. Mutant MTB strains lacking MctB exhibit copper accumulation and increased sensitivity to raised Cu^{+} levels, indicating MctB's crucial role in regulating copper export and maintaining optimal copper levels [72].

RicR, a copper-responsive transcriptional repressor, shares homology with CsoR. At low copper concentration, RicR binds to target promoters and suppresses gene transcription. However, when copper levels are elevated, RicR dissociates from DNA, inducing the expression of various genes including *mymT*, *lpqs* (encoding a putative lipoprotein), *rv2963* (encoding a putative permease), *socAB*, *mmcO*, as well as *ricR* [78]. Compared with CsoR, disrupting RicR has a more significant

regulatory effect on copper-stressed bacteria [79]. This suggests that MTB employs similar copper-specific sensors, CsoR and RicR, due to their different set points or sensitivities to copper in the cellular environment [80].

MymT is a cysteine-rich metallothionein capable of binding and sequestering up to six Cu^{+} ions within a thiolate core [81]. MymT detoxifies by redistributing intracellular metals and resisting oxidative stress. However, MymT mutant strains did not reduce virulence in mice, supporting the existence of different Cu resistance mechanisms in MTB [81].

Membrane-associated multicopper oxidase, MmcO, functions by catalyzing the conversion of the highly toxic form Cu^{+} to less toxic form Cu^{2+}, thereby providing resistance to copper toxicity. Additionally, it can scavenge ROS to mitigate oxidative stress induced by macrophages [82].



Zinc ions are depicted as green spheres, iron ions as red spheres, and copper ions as yellow spheres

Fig. 2 Various strategies employed by macrophages and MTB to maintain their metal ion homeostasis

5 Discussion

Mycobacterium tuberculosis, the pathogen of tuberculosis, continues to pose a threat worldwide. Scientists have developed four key antituberculosis drugs—isoniazid, rifampicin, ethambutol, and pyrazinamide, which are still the standard treatment for drug-sensitive TB. Due to the emergence of drug-resistant strains, it is imperative to develop new effective therapies. Multi-drug-resistant strains are resistant to both isoniazid and rifampicin, rendering many conventional treatments ineffective. Recently, three antituberculosis components have been incorporated into treatment regimens, which can effectively fight drug-resistant tuberculosis when used together [83]. However, the specific adverse effects and evolving resistance mechanisms have diminished their efficacy [83], highlighting the importance of developing a new therapeutic strategy.

Trace metal ions are essential for MTB because they participate in enzymatic reactions, reduce oxidative stress, and form proteins that facilitate

metabolism or constitute virulence during infections. Meanwhile, host cells, including macrophages, have developed numerous strategies to defend against pathogens by regulating the concentration of these metals. They employ nutritional immunity by limiting MTB's uptake of metals, while also accumulating excess metals in phagosomes as antimicrobial agents. To increase their survival rate under such strict restrictions, MTB has developed a sophisticated regulatory system to maintain metal balance. This includes the production of high-affinity metal ion chelators or importers to effectively acquire metals [6], and the utilization of high-affinity efflux pumps or proteins to secrete excess metals and inhibit metal uptake. Additionally, they can alter the form of metals, thereby alleviating the impact of oxidative stress [33].

Understanding the interaction of nutrition and immunity and elaborating the mechanisms of metal transportation are important for discovering novel therapies. Thus, we have summarized emerging treatments against MTB with the help of trace metals, offering insights for the discovery of new antituberculosis drugs.

5.1 Zinc therapy--zinc oxide nanoparticles

Metal combination therapies require new metal-delivering methods. The development of nanoparticle technology provides a way to create antimicrobial metal particles. Zinc oxide is an inorganic antimicrobial agent, which rarely leads to bacterial resistance [84]. The United States Food and Drug Administration (FDA) classifies it as a substance that is generally recognized as safe (GRAS). Therefore, ZnO is widely used in chemical, pharmaceutical and medical fields [15]. Zinc oxide nanoparticles (ZnO NPs) have a large surface area relative to their size and exhibit high catalytic activity. They are widely reported as agents with anticancer and antimicrobial properties [85].

ZnO NPs inhibit bacteria by directly destroying cell membrane, inducing ROS, and releasing zinc ions [16,86]. Although many studies confirm ZnO NPs' anti-TB properties against MTB and MTB-MDR, their full anti-TB capabilities and mechanisms require further research. ZnO NPs may attach to bacterial cell membranes, and then enter mycobacterium through endocytosis [84]. Their antimicrobial efficacy is affected by concentration and particle size, and smaller particles usually show higher activity due to their large surface area, which enhances the release of zinc ion and the production of ROS [15,87]. Additionally, smaller NPs can penetrate bacterial membranes more easily [15].

These properties suggest that ZnO NPs could be a promising strategy for treating drug-resistant tuberculosis. Krishna et al. produced 33 nm spherical particles and demonstrated that ZnO NPs have anti-tubercular effects, with MICs of 12.5 μg/mL against H37Ra and a selectivity index of over 10 (IC50/MIC) [84]. Heidary et al. fabricated spherical Ag NPs (5.4 ± 2.6 nm) and ZnO NPs (9.3 ± 3.9 nm), and found that Ag and ZnO NPs at 1 μg/mL could inhibit XDR strains, while Ag/ZnO NPs at 1-64 μg/mL could inhibit MDR and H37Rv strains [88]. Ag NPs enhance bacterial cell permeability by forming nanosized pores in bacterial membranes. Ellis et al. developed biodegradable multimetallic microparticles (< 4 μm diameter) containing Ag/ZnO NPs for direct pulmonary delivery, which increased rifampicin's potency against MTB by disrupting the cellular envelope [11]. Using an *in vitro* macrophage infection model, efficient uptake of these microparticles by MTB-infected THP1 cells was demonstrated. The intracellular release of Ag/ZnO NPs within the macrophage endosomal system enhanced rifampicin's potency by up to 76% by increasing membrane disorder in intracellular MTB. Mistry et al. observed a synergistic bactericidal effect when combining ZnO NPs with rifampicin, which showed significantly higher bacterial membrane

permeability and increased rifampicin uptake with the combination treatment [12]. Similarly, iron oxide nanoparticles enhanced the activity of antibiotics like rifampicin, isoniazid, and norfloxacin [89,90]. Lin et al. prepared ZnO/SeNPs, combining the antibacterial properties of ZnO and selenium, which showed strong efficacy against MTB [13]. Other combinations, such as Mg/ZnO NPs, also demonstrated synergistic anti-tubercular effects against multi-drug-resistant strains [14].

Traditional synthesis of ZnO NPs employs physical and chemical methods like co-precipitation, hydrothermal/solvothermal synthesis, sol-gel processes, microemulsion process and high-energy ball milling [91]. However, these methods often consume significant energy and produce toxic byproducts. Green synthesis using bacteria, fungi, or plant extracts to reduce zinc ions and form nanoparticles, offers an eco-friendly alternative [92]. Plant extracts with inherent antimicrobial properties can serve as stabilizers for ZnO NPs, which potentially enhance antimicrobial effects, biocompatibility, and anti-inflammatory properties while reducing toxicity [16].

ZnO NPs deposit within alveolar epithelial cells, triggering pulmonary inflammatory responses. Cho et al. intratracheally administered ZnO NPs (10.7 nm) at doses of 50 or 150 cm^2/rat to female Wistar rats and observed diverse pathological changes in the lungs, such as eosinophilia, regenerative goblet cell hyperplasia, and bronchocentric pulmonary fibrosis [94]. These adverse effects were attributed to zinc ions released from the nanoparticles. To date, ZnO NPs have not been successfully developed for clinical use against TB. Most studies remain in preliminary *in vitro* or *in vivo* stages.

While the exact mechanisms of ZnO NPs' anti-tubercular effects are still under investigation, their potential in combating multi-drug-resistant MTB strains is evident.

5.2 Traditional medicine: flavonoids on metal nutritional immunity

A large number of studies have shown that traditional Chinese medicine can alleviate the clinical symptoms of patients with pulmonary tuberculosis, but the relationship between the antibacterial mechanism and metal ions is still unclear.

Flavonoids, the largest group of plant-derived natural products, have been widely used as bactericides since ancient times because of their preventive and therapeutic effects on bacteria. They can suppress nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism. Additionally, flavonoids have been found to reduce bacterial adhesion and biofilm formation, affect porin on the cell membrane, change membrane permeability, and decrease pathogenicity, all of which are crucial for bacterial growth.

Flavonoids with the 6,7-dihydroxy iron chelation site (e.g., baicalein) are as potent as the clinically used deferoxamine in terms of iron chelation. Flavonols, which contain the 3-hydroxyl group, the 4-ketone group, and the 2,3-double bond, as well as the catecholic B ring, can exhibit iron chelation activity similar to deferoxamine. However, flavonoids with a 5-hydroxyl-4-keto chelation site have weaker iron chelation abilities [17].

Yadav et al. conducted experiments on 15 selected flavonoids and evaluated their antitubercular effects. The results showed that luteolin (MIC = 25 μg/mL) demonstrated the strongest antibacterial activity among all flavonoids tested. Quercetin, baicalein and myricetin showed comparable activity (MIC = 50 μg/mL), while hispidulin had the weakest effect (MIC = 100 μg/mL). The study revealed that hydroxyl groups at specific positions were crucial to inhibit MTB growth. These positions included 5,6,7 in ring A (as in baicalein), 3', 4' in ring B (as in luteolin, quercetin and myricetin), and 4' in the 5,7-dihydroxy-6-methoxy arrangement (as in

hispidulin). Furthermore, the research indicated that antitubercular activity requires two adjacent hydroxyl groups. However, when the hydroxyl group is methylated or glycosylated, as in casticin and hesperidin, the compounds lose their activity [93].

MTB can utilize the quorum-sensing (QS) system to form biofilms within the host as a defense against the host's immune system [94,95]. Baicalin, a flavonoid abundant in the traditional anti-inflammatory herb *Scutellaria baicalensis* Georgi, has iron-chelating properties. Its most effective iron-binding site arises from the C6, C7-dihydroxyl groups and a C2, C3-double bond in its structure [19]. Iron is crucial for microcolony formation and biofilm maturation in many pathogens. To prevent iron deficiency, bacteria produce siderophores, iron chelators that scavenge iron from the environment. Baicalin inhibits biofilm formation by suppressing the QS system, enhances the antibacterial activity of various antibiotics through a synergistic effect [96], affects bacterial cell growth and inhibits the bacterial beta-lactamase enzyme [97].

In MTB, infected macrophages form autolysosomes via the fusion of autophagosomes and lysosome [98]. MTB ingested by macrophages is destroyed there due to antimicrobial agents in autophagosomes like cathelicidin and ubiquitin [99]. To induce autophagy and suppress inflammation in the host, pathways such as MAPK activation and inhibitory activation of the PI3K/Akt/mTOR pathway are involved [100]. Baicalin induces autophagy and prevents inflammation by inhibiting the PI3K/Akt/mTOR pathway through the phosphorylation of Akt and mTOR.

Quercetin is a common dietary flavonoid. It combats *Mycobacterium spp*. through multiple mechanisms, including inhibiting key enzymes and blocking mycobacterial cell wall biosynthesis. In M. smegmatis and M. tuberculosis, quercetin specifically targets the subunit B of DNA gyrase [101]. Additionally, quercetin shows strong inhibitory effects and high affinity for the flavoenzyme uridine 5'-diphosphategalactopyranos emutase (UGM), an essential enzyme in MTB cell wall biosynthesis [102].

6 Conclusion

There are two primary mechanisms of drug resistance of MTB. One is that MTB possesses a lipid-rich cell wall due to mycolic acid, which interferes with the penetration of drugs into cells. The other is that MTB utilizes ATP-binding cassette (ABC) transporters and major facilitator superfamily (MFS) proteins to export antibiotics from the cytosol. The mechanism of metal nutritional immunity in the interaction between the host and Mycobacterium tuberculosis is complex and noteworthy. In this review, we aim to clarify the interaction mechanism of metal homeostasis regulation between MTB and the host. We also try to provide insights into the therapeutic strategies of tuberculosis. These methods include the use of zinc oxide nanoparticles as a potential anti-multi-drug-resistant-tubercular agent and the application of flavonoids such as baicalin, which have antitubercular and anti-inflammatory effects. The comprehensive investigation of the metal ion regulatory network in the future will help to develop new drugs against drug-resistant bacteria.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Credit authorship contribution statement

Tianyi Zhao: Writing – original draft, Writing – review & editing, Visualization, Investigation, Conceptualization. Qingxuan Wu: Writing – review & editing. Yihao Fan: Writing – review & editing. Ru Li: Writing – review & editing. Zifei Yang: Writing – review & editing. Wenxin Li: Writing – review & editing. Xiaoyun Zhao: Writing – review & editing, Investigation, Supervision, Conceptualization.

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