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Review

Metformin as a host-directed therapeutic in tuberculosis: Is there a promise?

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ABSTRACT

To complement the development of new or repurposed drugs for improving the treatment outcomes of drug-susceptible and drug-resistant tuberculosis, current insight also focuses on the use of host-directed therapy. Metformin, a drug often used in the management of type 2 diabetes mellitus, has attracted attention by virtue of its favourable activity as an adjunctive agent against tuberculosis, discovered through laboratory and clinical studies. To definitively establish its role as a host-directed therapeutic in tuberculosis, more preclinical and clinical research is still required to better delineate its mechanism(s) of action and optimal clinical use.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is still a major infectious disease globally. Furthermore, diabetes mellitus and human immunodeficiency virus (HIV) infection aggravate the formidable health challenge of TB, and so does antituberculosis drug resistance [1,2]. In 2017, an estimated 10 million people developed active TB worldwide, with 9% occurring in people living with HIV [1]. In the same year, an estimated 558,000 people developed rifampicin-resistant TB worldwide, with 82% being multidrug-resistant (MDR) TB [1]. Currently, the usual success rates for treating drug-susceptible TB and MDR TB, are 80–85% and 55–60%, respectively, in service programmes of many countries. The required length of treatment is 6 months for drug-susceptible TB, but this has to be considerably prolonged in the presence of drug resistance.

1.1. Rationale of host-directed therapy in tuberculosis

The host-microbe relationship in human TB is a very intriguing one. *Mtb* has inflicted morbidity and mortality in *homo sapiens* for an extremely long time, as revealed by the historical records. The pathogen is most astute, and its successful interaction with the host is readily recognised by its high prevalence of latent infection in mankind, through involving an estimated 23% of the world population [3]. Such an impressive infection rate due to *Mtb* is related to the adaptability of some tubercle bacilli to survive the immunological response/defence (both innate and adaptive) mounted by the host, including their assumption of a state of bacillary persistence, when the organisms are faced with various stress challenges, such as redox and pH changes, and antimicrobial administration. Thus, it is clearly warranted to focus dedicated efforts on the development of adjunctive immunotherapy, or more broadly host-directed therapy, to strengthen the arsenal for fighting TB [4,5]. Unveiling candidates for host-directed therapy against TB has been booming in the recent years, with identification of a number of potential drugs and agents, that already have documented value in the management of various medical diseases, such as cardiovasculopathy, arthropathy, diabetes mellitus and asthma [6,7]. Table 1 shows some important examples. In this short review, we only attempt to address the potential role of metformin (MET), a time-honoured biguanide for the management of type 2 diabetes mellitus, as a host-directed therapeutic in TB. This focusing is based on the ready availability of the drug and the recent surge in laboratory and clinical information regarding its possible role in the adjunctive therapy of TB. We highlight the crucial laboratory evidence and clinical data, and hope to submit perspectives regarding the promise (and limitation) of this drug in its contribution to the new paradigm of care for TB patients.

2. Mechanisms of metformin as a host-directed therapeutic in tuberculosis

In the recent past, there has been accumulation of some laboratory evidence pertaining to the underlying mechanisms of MET as a potential agent in host-directed therapy of TB. These data are briefly discussed as follows.

2.1. Macrophage activity

In an experiment that probably represented the pioneer work for

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Table 1
Some potential host-directed therapeutics in tuberculosis.

Name of compound	Currently approved clinical use
Ibuprofen	Arthritis and other conditions with pain
Etanercept	Rheumatoid arthritis and other arthropathies
Adalimumab	Rheumatoid arthritis
Metformin	Type 2 diabetes mellitus
Zileuton	Asthma
Statin	Hypercholesterolemia
Verapamil	Hypertension
Carbamazepine	Epilepsy and neuropathic pain
Valproic acid	Epilepsy
Desipramine	Depression
Imatinib	Chronic myeloid leukaemia and gastrointestinal stromal tumours
Bevacizumab	Some solid tumours
Pazopanib	Renal cell carcinoma

attempting to delineate the underlying mechanism(s) of MET as a host-directed therapeutic in TB, MET treatment was found to restrict intracellular growth of *Mycobacterium bovis*, and H37Rv strain as well as MDR strains of *Mtb* in the human monocyte/macrophage cell lines [8]. Such dose-related inhibitory activity of MET was found to be dependent on adenosine monophosphate-activated protein kinase (AMPK) signalling. Further elucidation of the mechanism(s) underlying mycobacterial growth inhibition has revealed that MET selectively induced the production of mitochondrial reactive oxygen species (mROS), likely through interacting with mitochondrial complex-1 in the host cells, overcoming the negative effect on synthesis of mROS furnished by the infecting mycobacteria. The resultant mROS load might result in apoptosis and eventually cell death. Addition of ROS-scavenging agents, such as glutathione and N-acetylcysteine abolished the induction of mROS by MET, alongside renewed intracellular growth of the tubercle bacilli.

2.2. Autophagy

In the pioneer study just discussed [8], aside from facilitating phagosome-lysosome fusion intracellularly, MET use also induced autophagy, but blockage of such activity did not appear to negate MET-mediated inhibition of mycobacterial growth. As there is now accumulating evidence regarding the possible roles of autophagy in killing or containment of *Mtb*, control of inflammation, and activation/augmentation of innate and adaptive immune response in the host [9,10], more work is required for ultimate delineation of such roles, especially in the context of potential effectiveness of MET in host-directed therapy of TB. Recently, MET was found to protect against doxorubicin-induced cardiotoxicity and spinal cord injury through mechanisms involving changes in autophagy regulation [11,12].

2.3. Cell-mediated immune response

Postulation regarding the protective or destructive result of granuloma formation in TB has driven investigations into the use of host-directed and specifically granuloma-targeted therapy to either promote natural immunity and healing or to limit the tissue damaging consequences of advanced granuloma formation [13]. Excessive pro-inflammatory responses are unfavourable because they can result in extensive tissue damage prior to the development of *Mtb*-specific adaptive immunity. MET adjunctive treatment was found to reduce the tissue pathology and to improve immune response in the afore-mentioned pioneer investigation [8]. Reduction of area of lung damage and granuloma formation, alongside increased lymphocytic infiltration, was observed histopathologically. MET-treated mice were found to have a trend of higher numbers of $CD4^+$ and $CD8^+$ T cells, including favourable results for the interferon-gamma secreting T cells. Clearly, further delineation of T cell phenotypes in greater details, notably Th1, Th2 and T regulators would be rewarding. In this [8] and other experiments [14,15], MET was consistently shown to reduce the expression of genes encoding cytokines and chemokines associated with inflammatory response, such as interleukin-1 beta, tumour necrosis factor-alpha, interleukin-6, and others such as monocyte chemoattractant protein-1 and intercellular adhesion molecule-1. Key signalling pathways involved likely include AMPK and nuclear factor kappa-B [14]. In a mouse model of bleomycin-induced pulmonary fibrosis, MET had favourable effects on the resolution of fibrosis in an AMPK-dependent manner [16]. Importantly, in a nonrandomised retrospective cohort of patients, the anti-inflammatory effects furnished by MET was shown irrespective of the presence or absence of diabetes mellitus [17].

2.4. Oxidative stress

In the past several years, there have been a number of studies addressing the MET-induced modulation of oxidative stress. Earlier on, an experiment has demonstrated that long-term MET administration in the male mice was associated with improvement of health-span and life-span [18]. At the molecular level, MET was found to increase AMPK activity and enhanced protection against oxidative stress with lessened chronic inflammation [18]. A more recent experiment has found a favourable role of MET regarding the control of oxidative stress, with corresponding change in the activities of catalase and superoxide dismutase (SOD), in the skeletal muscle of diabetic rats [19]. In a rat model of periodontitis, MET administration has also recently shown similar benefit, resulting in reduced inflammation and bone loss [20]. While upregulation of mROS was observed in the pioneer study regarding activity of MET against mycobacteria in macrophages [8], in cultured human umbilical vein endothelial cells, MET administration was however found to stimulate AMPK activity and inhibited hyperglycaemia-induced intracellular mROS production, alongside increased expression of manganese SOD mRNA [21]. In a randomised placebo-controlled study, MET use improved antioxidant status, enzymatic activity and inflammatory parameters in patients with type 2 diabetes mellitus [22]. In another clinical trial where diabetic patients were randomly assigned to receive MET or life style modification, the former intervention led to better results, regarding oxidative stress and antioxidant reserve in the host [23]. In a recent study, leukocytes from patients with type 2 diabetes mellitus were found to have enhanced levels of mROS and reduced mRNA levels of glutathione peroxidase 1 and sirtuin 3, compared to control subjects, as well as increase in leukocyte-endothelial interactions and levels of intercellular adhesion molecule-1 and P-selectin. All these afore-said changes were attenuated with MET therapy, suggesting protective effect of this biguanide against oxidative stress and leukocyte-endothelium interactions in diabetic patients [24]. MET was recently also found to protect against nephrotoxicity in diabetic subjects by reducing oxidative stress [25].

Oxidative stress is an important pathogenetic mechanism in type 2 diabetes mellitus, and it has been hypothesised, on reasonable grounds that such stress or more broadly disturbance in redox homeostasis, might contribute to the adverse outcomes of TB in diabetic patients [26]. Oxidative stress inherent to type 2 diabetes mellitus, especially in association with hyperglycaemia and immunological dysfunction, might increase the propensity for development of metabolically dormant *Mtb* persisters, with tolerance/recalcitrance to the bactericidal activity of antituberculosis drugs. Thus, delayed bacteriological conversion, higher failure/relapse, and greater mortality could be encountered as adverse TB treatment outcomes in diabetic patients. This hypothesis likely applies to other diseases/conditions with inappropriate oxidative stress, such as HIV infection [27].

Amelioration of oxidative stress might play a contributory role in the underlying mechanism(s) of MET as a host-directed therapeutic against TB. Research in this direction appears warranted, as currently there are little, if any, data. It is biologically plausible that the anti-

inflammatory activity and antioxidative capacity of MET might interact, with intricate cross-talk between various signalling and other pathways [28]. If modulation of oxidative stress is proven to be an underlying mechanism of MET as a host-directed therapeutic in TB, this might have significant implication in such adjunctive therapy, especially when associated with diseases with oxidative stress inherent to their pathogenesis, such as diabetes mellitus and HIV infection.

2.5. "Antimicrobial" activity

Interestingly, in an analysis using *in silico* systems, it was found that directional re-routing of metabolic fluxes through NAD *de novo* biosynthesis pathway and respiratory chain complex-1 (NDH-1) in *Mtb* furnished a possible alternative mechanism for ATP generation for the bacillary persisters [29]. As there appears structural and functional similarities for bacterial NDH-1 and mitochondrial complex-1, MET might be able to inhibit NDH-1 in *Mtb* in addition to its effect on the host mitochondrial complex-1. If that were the case, MET might be able to act on *Mtb* persisters via such "antimicrobial" mechanism [30]. Further fathoming may be indicated to unravel the mechanistic possibility. However, there appears to be limited enthusiasm so far to pursue in this direction.

3. Mouse experiments regarding the interactive effectiveness of antituberculosis drugs with metformin

A follow-up animal experiment by the pioneer investigators found the enhancement of activities of isoniazid (a key bactericidal first-line antituberculosis drug), and ethionamide (an important second-line antituberculosis drug) in acute and chronic models of murine TB, with the adjunctive use of MET [8]. However, not all animal studies definitively confirm the adjunctive role of MET in improving the chemotherapy effectiveness for TB. In a recent murine TB study involving the use of MET and first-line antituberculosis agents, only a trend towards better bactericidal activity against *Mtb* was observed, without achieving statistical significance [31]. Improvement of sterilising activity of the first-line drug regimen by MET was not shown, as gathered from the relapse proportions for different treatment durations. One possible reason for the disparity in the findings of this study [31] and those of the pioneer study [8] might be the different murine models used. Another possibility might be related to the experimental design, namely monotherapy [8] versus combination therapy [31] involving antituberculosis drugs. Furthermore, rifampicin use in this more recent study [31] might alter the pharmacokinetics of MET. One important determinant might be the organic cation transporters [32] for MET disposition, although other enzyme systems and transporters might also be mechanistically implicated. Recently, moxifloxacin was also found to be a potent inhibitor *in vitro* of disposition of MET and ethambutol, as mediated by organic cation transporters and multidrug and toxin extrusion proteins [33]. These potential drug-drug interactions would likely be of relevance in future animal experiments.

4. Current clinical data on tuberculosis outcomes with adjunctive use of metformin

The clinical data regarding TB outcomes associated with MET use are briefly reviewed as follows.

4.1. Latent infection due to Mycobacterium tuberculosis

In the clinical part of the afore-said pioneer study [8], evaluation of the findings of T-SPOT. TB test, an interferon-gamma release assay, in diabetic patients has suggested MET treatment might be associated with enhanced *Mtb*-specific T cell-mediated immune response for protecting the host against latent TB infection (odds ratio 0.44, 95% confidence interval: 0.20 to 0.95). In a case-control study performed retrospectively in India, there were 152 diabetic subjects with TB and 299 diabetic patients without TB [34]. Among case subjects, only 38/152 (25.5%) were on MET, whereas among control subjects, 171/299 (57.1%) were on MET. Poor glycaemic control (HbA1c greater than 8.0%) was found in 51.7% of case subjects and 31.4% of control subjects. The protective effect of MET against developing TB in diabetic subjects was found (odds ratio 0.256, 95% confidence interval: 0.16 to 0.40). In another recent retrospective, population-based cohort study in East Asia [35], diabetic patients classified as MET majors (with at least 60 days of MET use and less than 15 days of sulfonylurea use) had a significantly lower risk of having active TB (adjusted hazard ratio 0.477, 95% confidence interval: 0.268 to 0.850), with dose-dependent effectiveness of the biguanide. Glycaemic control was not a significant confounding variable, and adjustment was also made for statin use as a confounder. In a similarly performed study in the same locality, MET administration was found to be independently associated with a reduced risk of active TB (adjusted relative risk 0.24, 95% confidence interval: 0.18 to 0.32) [36], thus corroborating the results of the other cohort study [35].

4.2. Tuberculosis disease

Furthermore, in the clinical section of the pioneer report [8], subjects with type 2 diabetes mellitus not receiving MET appeared more likely to have advanced pulmonary disease radiographically (odds ratio 1.307, 95% confidence interval: 0.77 to 2.2). Diabetic patients in MET group had fewer cavities than those in non-MET group (odds ratio 0.60, 95% confidence interval: 0.36 to 0.97). Multivariate analysis also revealed diabetic patients with TB in the MET group were less likely to die (log odds ratio, −1.75, 95% confidence interval: -3.14 to −0.36). In a retrospective cohort study undertaken in South Korea, 105 diabetic patients with culture positive TB were evaluated; 62 patients received MET and 43 patients did not [37]. Baseline characteristics, except for the presence of chronic renal disease and statin use, were not significantly different between the MET and non-MET groups. MET treatment had no significant effect on sputum culture conversion after 2 months of TB treatment and the TB recurrence rates within one year post-treatment. However, among diabetic patients with cavitary disease, the proportion of culture conversion after 2 months of TB treatment was significantly higher in the MET group (odds ratio 10.8, 95% confidence interval: 1.22 to 95.63) [37]. In another retrospective cohort study, 2416 patients with TB were assessed [38]. After adjusting for age, sex, chronic renal disease, malignancy, hepatitis C, tobacco smoking, cavitary disease and treatment adherence, patients with diabetes mellitus were more at risk of death during TB treatment (odds ratio 1.91, 95% confidence interval: 1.51 to 2.40), compared to patients without diabetes mellitus. Diabetic patients also had a higher risk of remaining culture-positive after 2 months of therapy (odds ratio 1.72, 95% confidence interval 1.25 to 2.38). Multiple confounding factors notwithstanding potentially, diabetes mellitus increased the risk of adverse treatment outcomes for TB. MET use was found to be significantly associated with decreased mortality of diabetic patients during their antituberculosis therapy (hazard ratio 0.56, 95% confidence interval: 0.39 to 0.82), in the face of similar or worse glycaemic control [38]. In a retrospective analysis of 58 culture-positive pulmonary TB patients with type 2 diabetes mellitus in China [39], there were only 16 (27.6%) patients on MET. No significant difference in glycaemic control between the MET and non-MET groups was found, but the TB treatment success rate and the 2-month sputum culture conversion rate were higher among patients on MET treatment than those without the biguanide treatment, namely 93.8% versus 71.4%, and 87.5% versus 71.4%, respectively. Furthermore, the relapse rate of tuberculosis over a 3-year follow-up period after treatment was 6.3% in the MET group versus 35.7% in the non-MET group (P = 0.045) [39].

Table 2
Reported tuberculosis outcomes with adjunctive use of metformin.

Outcomes	References
Protection against reactivation of latent infection to active disease	[8,34–36]
Less pulmonary cavitation	[8]
Trend towards less advanced pulmonary disease	[8]
Increased proportion of 2-month sputum culture conversion to negativity	[37,39]
Decreased patient mortality	[8,38]
Decreased disease relapse	[39]

5. Future clinical evaluations regarding adjunctive use of metformin in tuberculosis

A number of favourable messages regarding treatment outcomes of TB concurrent with the use of MET, have emerged from clinical studies in different parts of the world (Table 2), but it is important to note that all the studies/trials heretofore published are retrospective in nature. In order to delineate the definitive role of MET as an adjunct to anti-tuberculosis chemotherapy, against both drug-susceptible and drug-resistant tuberculosis, prospective randomised controlled clinical trials do appear imperative. Well-designed prospective cohort studies with well-defined populations would also help. Aside from ascertaining unequivocally the effectiveness of MET as a host-directed therapeutic in different forms of TB, the possibility of drug-drug interactions, as alluded, should be addressed, and so are the potential adverse reactions or toxicities induced MET alone, or through pharmacokinetic/pharmacodynamic interaction with the conventional and/or new anti-tuberculosis drugs. Some of the toxicities of MET are common but mild, whereas others are rare but severe. An example of the former is gastrointestinal disturbance [40,41] and that of latter is lactic acidosis [42,43]. Thus in the design of the studies, the dose of MET evaluated would be critical, especially in terms of its metabolic and toxic effects, as well as those pertaining to immunomodulation (and other host-directed activities beyond) [10]. Underlying cardiac, renal and hepatic functions of the patients also contribute to fathoming of rational dose(s) for them in these clinical trials [43].

6. Epilogue

Many questions still have to be answered through laboratory and clinical research before the role of MET can be firmly established as a host-directed therapeutic against TB. With guarded optimism, obstacles to hurdle might be anticipated in the road of development of this potentially promising agent for approved clinical use.

Conflicts of interest

KC Chang, DP Chan and Y Zhang declare no conflicts of interest.

WW Yew was consultant to Otsuka Pharmaceutical Company until July 2016.

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