Quantitative pharmacology approaches to inform treatment strategies against tuberculosis

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Chapter 8

Quantitative systems pharmacology modeling framework of autophagy in tuberculosis: application to adjunctive metformin host-directed therapy

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Abstract

Background: Quantitative systems pharmacology (QSP) modeling of the host-immune response against Mtb can inform rational design of host-directed therapies (HDTs). We aimed to develop a QSP framework to evaluate the effects of metformin-associated autophagy-induction in combination with antibiotics.

Methods: A QSP framework for autophagy was developed by extending a model for host-immune response to include AMPK-mTOR-autophagy signalling. This model was combined with pharmacokinetic-pharmacodynamic models for metformin and antibiotics against Mtb. We compared the model predictions to mice infection experiments, and derived predictions for pathogen and host-associated dynamics in humans treated with metformin in combination with antibiotics.

Results: The model adequately captured the observed bacterial load dynamics in mice Mtb infection models treated with metformin. Simulations for adjunctive metformin therapy in newly diagnosed patients suggested a limited yet dose-dependent effect of metformin on reducing the intracellular bacterial load when overall bacterial load is low, late during antibiotic treatment.

Conclusions: We present the first QSP framework for HDTs against Mtb, linking cellular-level autophagy effects to disease progression and adjunctive HDT treatment response. This framework may be extended to guide the design of HDTs against Mtb.

Introduction

The increasing burden of *Mycobacterium tuberculosis* (Mtb) infections is a major global health concern associated with approximately 1.5-2 million deaths annually[1]. Current first-line treatment to active tuberculosis (TB) disease, include a two-month intensive phase with rifampicin, isoniazid, pyrazinamide, and ethambutol followed by a four-month continuation phase with rifampicin and isoniazid (HRZE). A long treatment duration, common treatment failure, relapse, and emergence of multi-drug resistant Mtb strains are key challenges to successful TB treatment[2].

Host-directed therapies (HDT) aim to exploit the interplay between the pathogen and the host immune response[3,4]. HDTs are increasingly being studied for treatment against Mtb infections. One of the most studied HDT strategies to date is autophagy induction[5]. Autophagy is an intracellular catabolic process involving delivery of excessive or damaged cellular components, including bacteria, to lysosome for degradation to maintain homeostasis. The AMPK-mTOR signaling pathway is an important regulator of autophagy. Mtb activates phosphorylation of Akt, which stimulates phosphorylation of mTORC1. Activation of mTORC1 inhibits autophagy by phosphorylation of various autophagy-related proteins[6]. Preclinical studies have demonstrated involvement of mTOR signaling pathway in the host response to Mtb, suggesting its relevance as therapeutic target[6,7]. Therefore, metformin, an antihyperglycemic agent and mTORC1 inhibitor, has been proposed as potential HDT against Mtb[6,7]. Metformin adjunctive therapy in diabetic TB patients was found to be associated with an improved therapy success rate and lowered mortality[8,9].

Quantitative systems pharmacology (QSP) models aim to capture mechanistic details of the interactions between a biological system and pharmacokinetic-pharmacodynamic (PKPD) properties of a drug[10]. QSP-based characterization of drug-host-pathogen interactions may allow evaluation of expected treatment responses upon perturbation of specific targets, which may help to identify promising HDT targets and to evaluate different potential combination treatment strategies. Within the TB field, the mathematical modeling approaches have primarily focused on PKPD modeling focusing mostly on design of antibiotics [11,12]. In addition, multiscale systems biology models of the host-immune response in response to Mtb infections have been developed[13,14]. The prior immune response model[13] have been combined with PKPD models of HRZE to explore the impact of patient immune response on the treatment outcomes[15–17]. Any of these models did not include HDT relevant pathways; however, established a strong basis for further developing QSP framework to enable design of HDTs.

To guide design and development of HDTs, relevant HDT pathways must be added to the QSP framework. The autophagy-regulating AMPK/mTOR pathway represents an important factor for HDTs. There are currently no mathematical models available in literature describing mTOR signaling-mediated autophagy in TB. The objectives of this work were, (1) to develop a QSP framework of host-immune response including autophagy-mediated interactions, and (2) to evaluate the effects of metformin-associated autophagy-induction in combination with HRZE treatment.

Methods

The developed QSP framework **(Figure 8.1)** included, (1) PK models for standard antibiotics and metformin, (2) TB host-immune response model including PD effects of HRZE, and (3) autophagy model including PD effects of metformin. The QSP model development was facilitated by adaptations of various models presented in literature[17–19]. A set of ODEs describing dynamics of intra- and extra-cellular bacteria in host lungs as functions of time, and dynamics of immune-response components, such as macrophages, cytokines, and lymphocytes as functions of time and bacterial load, form the core of our QSP framework [18]. The core model was then linked to a model describing the dynamics of AMPK-mTOR signaling proteins leading to autophagy[19]. The interactions between Mtb and autophagy connects these two models. Moreover, the combined TB host-immune response-autophagy model was linked with models capturing PKPD relationships of HRZE and metformin.

Model development

The details of the model development process are provided in supplementary materials (S8.1), and the key steps are presented below.

Pharmacokinetics

PK models of four antibiotics, HRZE, were reproduced from literature-based population PK models[20,21]. Plasma concentrations of HRZE following standard of care dosing were simulated using the PK models. HRZE intra- and extra-cellular lung concentrations were predicted by applying plasma to lung alveolar cells and plasma to lung epithelial lining fluid ratios respectively obtained from literature[22]. To predict lung concentrations of metformin, we developed a minimal physiologically based PK model for metformin including a lung compartment[23] (S8.1.1).

TB host-immune response and pharmacodynamics of standard antibiotics

A published model that captured the dynamics of the host-pathogen interactions following Mtb infection was implemented[18]. This host-immune response model contained host-pathogen interactions in lungs, and included three population of macrophage (resting-, activated-, and infected-), various cytokines (IFN-γ, TNF-α, IL-10, IL-4, IL-12) and lymphocytes, as well as intra- and extra-cellular Mtb populations. An update was made to this model to add the turn-over of IL-1b and IL-1b-mediated bacterial elimination[24] (S8.1.2).

We included two Mtb growth phases, fast and slow, as a simple implementation of initial rapid progression of active disease[13,18]. The switch from fast to slow growth rates was empirically set to 21 days post-infection based on mice infection experimental results[25,26]. We used the slow phase bacterial growth rate estimates same as the growth rate values from the reproduced TB host-immune response model[18]. The growth rates for the initial fast phase were optimized using digitized data from mice Mtb infection experiment[25](S8.1). Bactericidal effects on intra- and extra-cellular bacterial population and bacteriostatic effects on growth rates of bacteria driven by intra- and extra-cellular lung concentrations of HRZE were reproduced from the literature[17].

Autophagy and pharmacodynamics of metformin

The AMPK-mTOR cell signaling network model from literature was reproduced[19]. This model captured the dynamics of key proteins involved in AMPK-mTOR signaling pathway and includes relative interactions between proteins involved in AMPK-mTOR signaling pathway, such as, insulin receptor substrate, class I phosphatidylinositol 3-kinases, AMPK, mTORC1, and mTOR complex 2 (mTORC2). This model was updated to include various Mtb- and autophagy-related components. The updates can be categorized into: (1) the effect of Mtb Infection on autophagy inhibition due to activation of AMPK-mTOR signaling and (2) the effect of autophagy of Mtb elimination. Gene AKT3, a key upstream regulator of AMPK-mTOR signaling pathway, was found to be induced 1.38-fold in Mtb-infected vs. uninfected mice based on differential expression in lungs of Mtb infected vs. uninfected mice[6]. This ratio was added as a proportional scaling factor in the model on production of AKT to simulate the presence of Mtb and its impact on key down-stream proteins involved in AMPK-mTOR signaling, including mTORC1 (S8.1.3). Due to the limited data availability, time-course effects of progression of Mtb infection on autophagy is not included in the current model.

The effects of AMPK-mTOR signaling on autophagy were model using a direct effect saturable Emax model. Autophagy at time of Mtb infection was set to 100 % to represent a healthy state prior to infection. Then, the percent inhibition of autophagy due to Mtb infection and subsequent AMPK-mTOR signaling activation modeled. Next, the autophagy model was combined with TB host-immune response model by introducing autophagy-mediated intracellular bacterial killing and autophagy-mediated extracellular to intracellular bacterial uptake. These processes were incorporated as first-order processes, and the parameters were informed by Mtb survival data from in vitro infection experiments with and without metformin treatment[27](S1.3). The inhibitory effect of metformin on mTORC1 phosphorylation was incorporated using an indirect effect saturable Emax model, and the parameters were obtained from the literature[28,29].

Model evaluations

The combined TB-Autophagy QSP framework predictions were first compared to observed digitized lung bacterial load data from untreated and metformin-treated mice infected with Mtb[27]. To this end, the QSP model was scaled from humans to mice by applying volume differences between the species. To evaluate HRZE PKPD components of the combined QSP model, the predicted change in bacterial load over time after start of HRZE treatment was compared against reported values for TB patients[30–32].

Sensitivity analysis

High uncertainty existed in some parameters, especially for parameters related to autophagy model due to limited data availability. To further understand the impact of uncertainty in the parameters on model predictions, global uncertainty and sensitivity analysis using Latin hypercube sampling (LHS) and partial rank correlation coefficient method using 500 samples was performed[33,34]. The outcome used in this analysis was predicted total bacterial load. All parameters, except the PK and PD parameters, were evaluated in the global uncertainty and sensitivity analysis. The parameters ranges used for LHS were the same as the previous model for TB host-immune response model components and were varied by 20% for autophagy-related components[18].

Simulations of metformin-associated autophagy induction in humans

Typical TB patient simulations were conducted using the QSP framework to predict the effects of autophagy induction with metformin on overall treatment outcome. Typical TB patient simulations were performed using parameter values presented in S8.2. A typical virtual TB patient was defined as a 70 kg human. No random effects or uncertainty components were included in the simulations. An initial extracellular Mtb inoculum of 100 bacteria was introduced at day 0 in all simulations.

First, the simulations were performed to evaluate effects on bacterial load and on cytokine levels following HRZE therapy with and without adjunctive metformin treatment at three different dosing regimens starting at day 180 post-infection, i.e., upon diagnosis. Day 180 post-infection was selected as the approximate time to diagnosis and as such starting point for treatment based on prior model[17]. In these first set of simulations, metformin was added at the same time as starting HRZE treatment. Additional simulations were performed to predict the effects on total bacterial load if metformin was added at end of two months intensive HRZE treatment. Metformin dosing regimen used in the simulations included 250 mg, 500 mg, and 1000 mg, all BID. HRZE regimen in the simulations included 300 mg isoniazid, 600 mg rifampin, 1500 mg pyrazinamide, and 1100 mg ethambutol all QD for 2 months, followed by the same dose of isoniazid and rifampin for 4 months. Next, to understand the effects of metformin on the TB disease progression in scenarios where diabetic patients would be receiving metformin for their glycemic control at the time of infection with TB[8,9], simulations were performed to predict the effects of 500 mg BID metformin treatment starting at day 1 post-infection.

Software

All parameter optimization and model simulations were conducted in R and RStudio using nlmixr and RxODE packages[35]. Literature model for autophagy was converted from SBML file to ODEs in R using IQRsbml package [https://iqrsbml.intiquan.com/main.html].

Results

The QSP framework included combined host-pathogen interactions model, AMPK-mTORC1 signaling pathway model including autophagy, and PKPD models of HRZE and metformin **(Figure 8.1)**.

The QSP framework simulations recapitulate observed in vivo response to metformin

The model was evaluated by comparing predictions to the observed data. The model predictions for total bacterial load showed good agreement with observed digitized lung bacterial load data from untreated mice infected with Mtb[6] **(Figure 8.2A)**. The simulations with standard TB therapy starting at day 180 post-infection in TB patients predicted previously reported change in bacterial load from baseline with HRZE treatment reasonably well **(Figure 8.2B)**. Overall, these assessments suggested the reliability of the model for the objectives of this analysis.

Sensitivity analysis provides insights into the mechanistic details of the infection

The global uncertainty and sensitivity analysis suggested that the bacterial load was more sensitive to the parameters of host-pathogen interaction model compared to those of the autophagy model (S8.3). In general, the host-pathogen interaction model parameters that correlated with the bacterial load the most were related to macrophage recruitment, macrophage activation or deactivation, phagocytosis, IFN-y production, or IL-1b- or FAS-FAS-mediated apoptosis. Most of these parameters were identified in the sensitivity analysis in the prior models too[18]. In the prior models, these parameters were obtained either from literature or were estimated using in vitro or mice experiments' data and therefore, are considered relatively reliable. One parameter related to the autophagy model, AKT dephosphorylation rate, was found positively correlated with the bacterial load, and thus negatively correlated with infection control. This highlights the key role of Mtb evasion and inhibition of autophagy on disease progression. This parameter was unchanged in the current model from the previous AMPK-mTOR signaling model. In the previous work, this parameter was estimated using experimental data from immunoblots and thus deem reliable. This sensitivity analysis given uncertainty in the parameters provide a thorough picture of the current state of the model (Figure S8.3).

Figure 8.1 Combined TB-Autophagy QSP framework. The model captures the dynamics of host-immune response in the lungs because of Mtb infection. The model consists of various species of macrophages, lymphocytes, and the key cytokines involved in both innate and adaptive immune response against Mtb. The model includes the growth of Mtb as well as immune-mediated elimination of Mtb affecting the overall Mtb population. The immune-mediated bacterial killing include mainly cytokine- and lymphocytes- mediated apoptosis as well as autophagy. The model also consists of Mtb evasion mechanism, such as, induction of AMPK-mTOR pathway and inhibition of autophagy. Bi=intracellular Mtb, Be=extracellular Mtb, Ma=activated macrophage, Mi=infected macrophage, Mr=resident macrophage, T80=precursor-activated CD8+ T cells, T8=sub-class (IFN-y producing) of activated CD8+ T-cells, Tc=subclass (cytotoxic lymphocytes) of activated CD8+ T-cells, Th0=naïve T-cells, Th1=type 1 helper T-cells, Th2=type 2 helper T-cells. Figure created with biorender.com

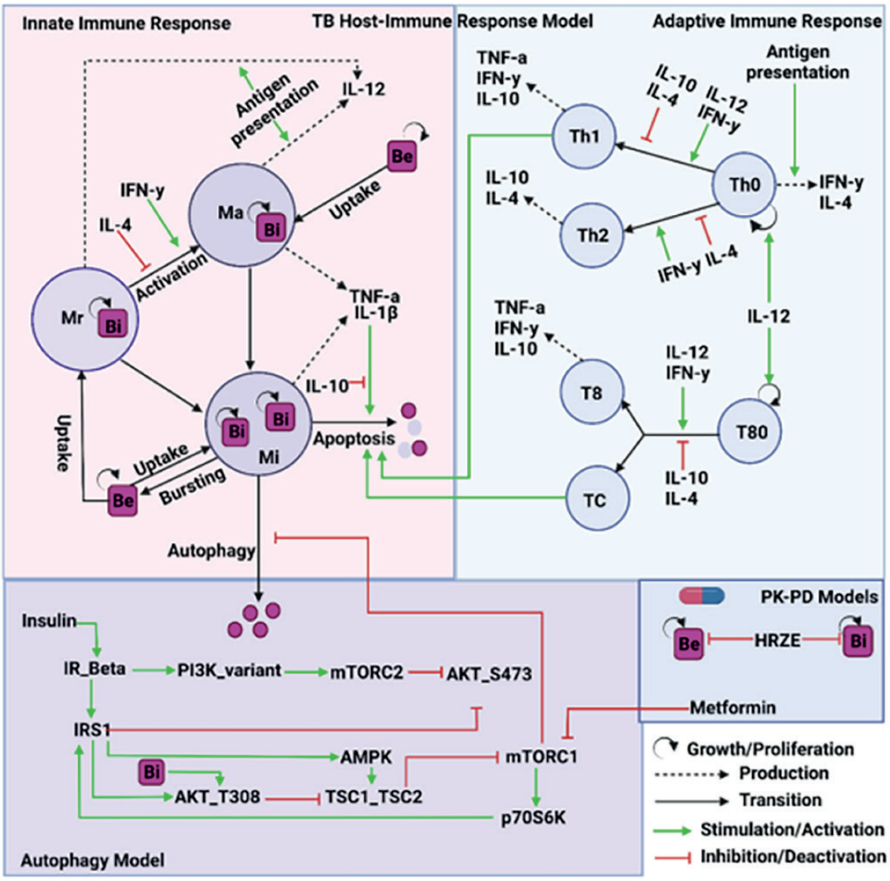
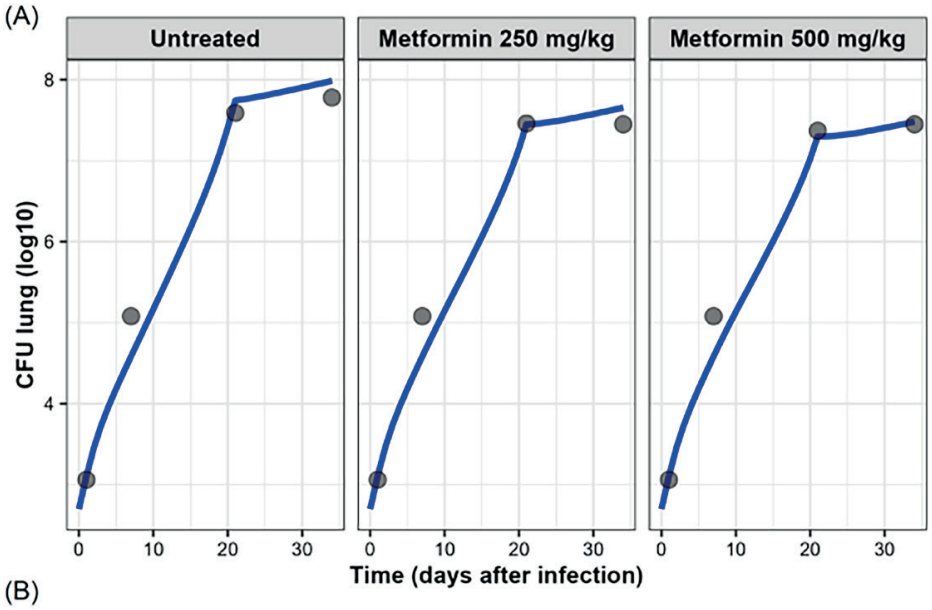


Figure 8.2 Time course of predicted and observed lung bacterial load: (A) Mtb-infected mice treated or untreated with Metformin, (B) Tuberculosis patients treated with standard antibiotic regimen. The model predictions for total bacterial load agree well with the observed mice data treated with or without metformin. Additionally, model predictions for effects of standard antibiotics therapy reasonable agree observed change in bacterial load from baseline data from TB patients. CFU=colony-forming unit, Metformin was administered daily from day 7 through 35 with six days on, one day off regimen in mice, points represent observed and lines represent model predictions.



Metformin-associated autophagy induction is predicted to provide dose-dependent reduction in intracellular bacterial load

The simulations for TB disease progression, i.e., prior to the start of treatment suggested that Mtb infection is predicted to reduce autophagy by 55% in a typical subject. We compared the effects of HRZE with or without adjunctive metformin treatment on bacterial load and cytokine levels in a typical virtual TB patient. These simulations considered a typical scenario where the treatment was started upon diagnosis of TB, which was considered around day 180 after initial infection. Adjunctive metformin with HRZE treatment was predicted to show limited yet apparent dose-dependent increase in autophagy-mediated intracellular and total bacterial elimination when total bacterial burden is relatively low after first two months of treatment **(Figure 8.3a)**. No significant impact was predicted on cytokine levels in the adjunctive metformin with HRZE scenario compared to HRZE only scenario **(Figure 8.3B)**. The simulations for a scenario where metformin was added two-months after the start of intensive phase treatment with HRZE predicted that adjunctive metformin may reduce overall treatment duration by 3-5 days (Figure S8.4). Overall, we conclude that adjunctive metformin treatment may provide modest benefit in reducing Mtb bacterial load in TB patients during the continuation phase of HRZE treatment.

Metformin may delay disease progression in diabetic tb patients

We assessed if metformin would delay TB disease progression if metformin was administered prior to TB diagnosis, i.e., in scenarios where diabetic patients would be receiving metformin for their glycemic control at the time of infection with Mtb **(Figure 8.4a)**. For these simulations, metformin input was added at the same time as initial bacterial infection. We found that metformin use in diabetic TB patients would delay TB disease progression as assessed by intra-, extra-, and total-bacterial load. Lower levels of pro-inflammatory cytokines, IL-1b, TNF-α, IFN-y, and IL-12, were also predicted in metformin-treated vs. no metformin-treated typical patient **(Figure 8.4b)**. Overall, these simulations suggest some protective effects over tissue damage of metformin use in diabetic TB patients. As these simulations represent scenarios prior to TB diagnosis, thy do not provide guidance in metformin treatment for TB patients. However, these simulations provide mechanistic insights into the role of autophagy on the dynamics of TB infection.

Figure 8.3 Typical patient simulations for adjunctive Metformin to atandard antibiotics treatment at various dosing regimen starting at day 180 post-infection: (A) Bacterial load, (B) Cytokines. The simulations suggest dose-dependent effects of metformin on reduction of intracellular bacterial load. The reduction in intracellular bacterial elimination with adjunctive metformin treatment; however, does not significantly affect extracellular and thus total bacterial load compared to standard antibiotics only treatment when total bacterial burden is high. HRZE refers to 2 months of rifampicin, isoniazid, pyrazinamide, and ethambutol + 4 months of rifampicin and isoniazid regimen; vertical dashed line refers to end of 2 months regimen.

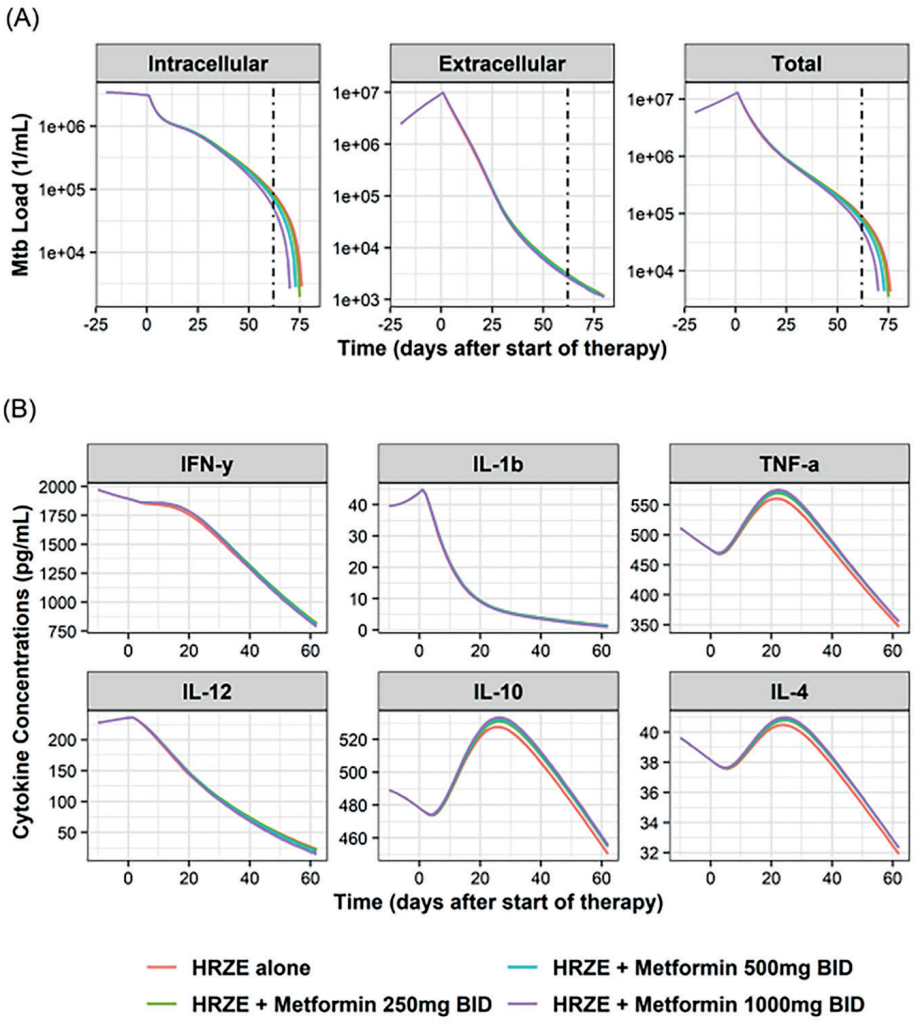
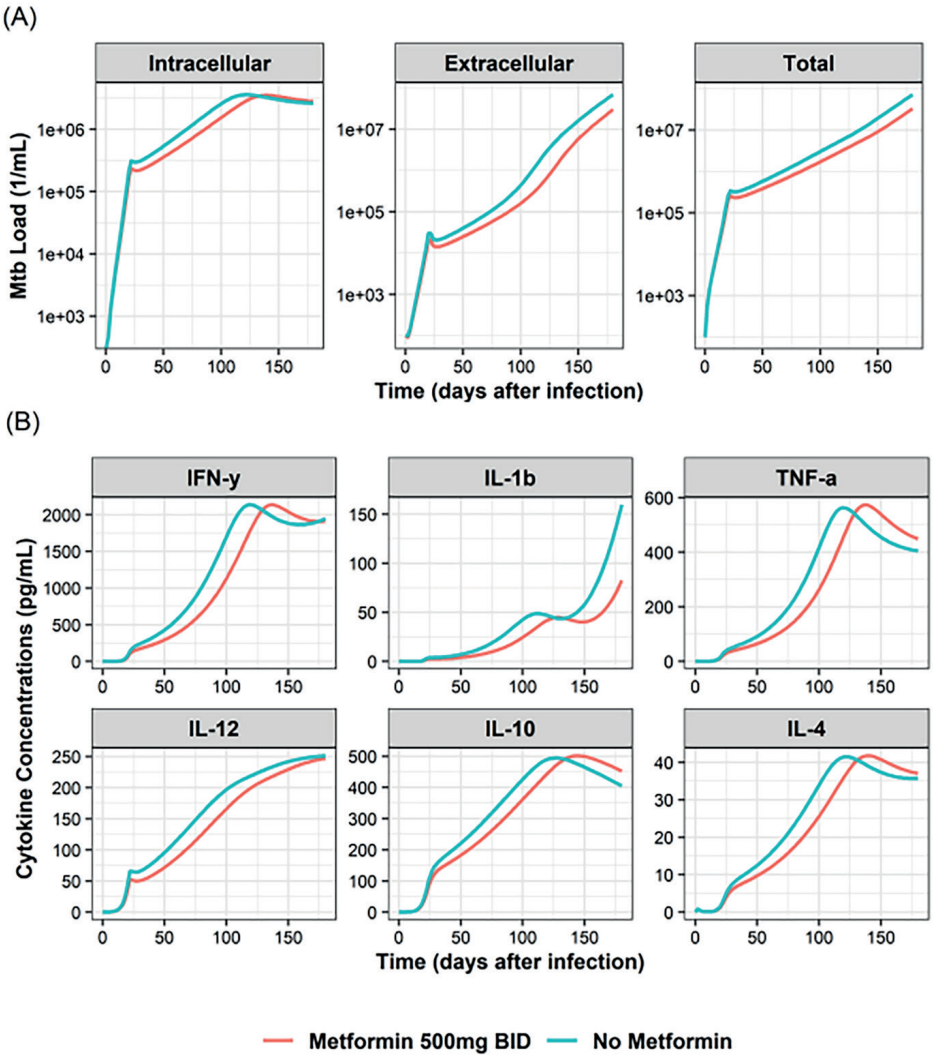


Figure 8.4 Typical patient simulations with or without 500 mg twice daily Metformin starting at day 1 post-infection: (A) Bacterial load, (B) Cytokines. The model predicted some benefits of metformin use in delaying the disease progression in virtual diabetic patients receiving metformin as compared to non-treated patients.



Discussions and conclusions

Here, we developed a first QSP framework for design and evaluation of HDTs focusing on autophagy. Our model was able to recapitulate results from an in vivo study evaluating metformin as a HDT in Mtb-infected mice. We applied the framework to predict treatment effects of autophagy induction by metformin in a typical TB patient.

Our analysis identified a modest beneficial effect of adjunctive metformin treatment in a typical TB patient, after intensive phase antibiotics treatment when total bacillary load is predicted to be relatively low. The predictions suggested that overall effects of treatment with metformin would depend on extracellular-to-intracellular bacteria ratio, which may depend on the stage of infection. The model also predicted some benefits of metformin use in delaying the disease progression in virtual diabetic patients receiving metformin. Our results agree with the clinical reports where lowered mortality rates were reported in diabetic patients receiving metformin[8,9]. A key Mtb survival strategy depends on provoking a non-sterilizing immune response, allowing for Mtb to replicate beyond reach of most immune mechanisms. As part of host-pathogen interactions, granuloma formation limits Mtb growth, but also provide niche for replication by disseminating Mtb to other areas[36]. Metformin, and HDTs in general, may provide beneficiary effects early after initial infection, i.e., in newly infected TB household contacts, or late during treatment, i.e., after sputum has been sterilized but when small numbers of persisting bacteria are still present. In these scenarios, small changes in the survival of a rather small bacterial population may have a large effect on infection outcome, and future studies may consider evaluating this.

Our model provides relevant quantitative insight into the mechanistic details of factors contributing to autophagy-mediated bacterial elimination. Lack of predicted effects of metformin at doses up to 1000 mg BID on total bacterial load can also be attributed to its potency on AMPK-mTORC1-autophagy signaling and its distribution in lungs, in addition to extracellular-to-intracellular bacterial ratio. Previously, metformin dose-ranging study that evaluated effects of metformin at doses 100–10000 uM on Mtb survival in human monocyte-derived macrophages showed no increased Mtb survival at doses up to 500 uM. In the same study, approximately 4% reduction in total bacterial load on day 35 was noted in mice treated with 250 mg/kg and 500 mg/kg metformin daily from day 7-35 (6 days on, 1 day off)[6]. Our mPBPK model predicted mice lungs Cmax 668 uM and 1336 uM in 250 mg/kg and 500 mg/kg metformin dose groups, respectively. When these body weight-based

doses of metformin that were evaluated in mice are compared to clinically feasible doses, predicted lungs Cmax in humans are approximately 10- to 15-fold lower than those predicted in mice. As such, it would be no surprise that our predictions showed no significant effects of metformin on the reduction of bacterial load. In fact, a recently completed clinical trial evaluating adjunctive metformin treatment to standard treatment in TB patients reported that metformin treatment did not significantly reduce time to sputum conversion as compared to controls[37].

The integrated QSP framework connects the complex intracellular process, autophagy, to disease outcome at organism-level. The model can be easily adapted to perform evaluations of other mTORC1 inhibitors mTORC1-independent autophagy inducers in the future using similar approach as ours. Some candidate drugs include, everolimus, statins, PI3K inhibitors, and tyrosine kinase inhibitor. The model can also facilitate in silico evaluations of perturbations of various proteins involved in autophagy and predict their effects on the outcome, as such enable target identification for optimal autophagy induction. For example, the model may be used in combination with screening assays to prioritize further development of potential HDTs.

One of the limitations of our model was that it built upon a prior relatively simple TB host-immune response model[18]. In our model, we added empirical transition from fast- to slow- Mtb growth phase to resemble initial log-phase increase in bacterial load. However, growth and treatment effects on different subpopulations of Mtb, i.e., non-persisters and persisters, at a given time is not included in the model. Future work may integrate a mechanistic model with various subpopulations of both intra- and extra-cellular Mtb into the QSP framework. For this, measurements of various Mtb subpopulation from sputum or bronchoalveolar lavage fluid of TB patients are required. The proposed integration may also require applying antibiotics' bacterial kill rates specific to the subpopulation. In general, the current construct of TB host-immune response model is relevant for our primary objective, i.e., to evaluate different treatment scenarios with and without metformin in TB patients when bacterial load has already reached relatively high.

Our model includes relative activity of the key proteins involved in the AMPK-mTOR-autophagy signaling, and however, do not consider total concentrations of these proteins. The original data-driven AMPK-mTOR model that was adapted in this work was developed using immunoblot data from HeLa cells, and therefore considered relative activity of the proteins[19]. Direct measurements of these are not available to date. As such, uncertainty exist in parameters impacting effects of

AMPK-mTOR signaling on autophagy and treatment effect predictions. However, the current approach of using relative activities of the AMPK-mTOR signaling proteins to evaluate their downstream effects on autophagy provides a useful alternative in absence of absolute proteins data.

To summarize, we developed a QSP framework for autophagy-inducing HDT by integrating a previously developed models for AMPK-mTOR signaling, host-pathogen interactions, and PKPD. We extended the framework to include autophagy to enable in silico evaluations of adjunctive metformin to antibiotics in TB patients. Our predictions suggest that metformin may provide some beneficiary effects when overall bacterial load, or extracellular-to-intracellular bacterial ratio is low. Overall, this is the first QSP that links cellular-level events affecting autophagy to disease progression and may further be developed to guide HDT design and development for treatment of TB.

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Supplementary Materials

The supplementary material can be accessed from the following GitHub repository: https://github.com/krinaj/TB_Autophagy_Metformin_Model