

Metformin Increases Cell Viability and Regulates Pro-Inflammatory Response to Mtb

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Introduction: Current TB treatment regimens are pathogen-directed and can be severely compromised by the development of drug resistance. Metformin has been proposed as an adjunctive therapy for TB, however relatively little is known about how metformin modulates the cellular interaction between Mtb and macrophages. We aimed to characterize how metformin modulates Mtb growth within macrophages.

Methods: We utilized live cell tracking through time-lapse microscopy to better understand the biological effect of metformin in response to Mtb infection. Furthermore, the potent first-line anti-TB drug, isoniazid, was used as a comparator and as a companion drug.

Results: Metformin caused a 14.2-fold decrease in Mtb growth compared to the untreated control. Metformin combined with isoniazid controlled Mtb growth is slightly better than isoniazid alone. Metformin demonstrated the ability to regulate the cytokine and chemokine response over a 72 hour period, better than isoniazid only.

Conclusion: We provide novel evidence that metformin controls mycobacterial growth by increasing host cell viability, and a direct and independent pro-inflammatory response to Mtb. Understanding the impact of metformin on Mtb growth within macrophages will advance our current knowledge on metformin as an adjunctive therapy, providing a new host-directed approach to TB treatment.

Keywords: metformin, tuberculosis, host-directed therapy, INH, adjunctive therapy

Introduction

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB), a communicable disease that is a major cause of ill health, high global morbidity and mortality rates and the leading cause of death from a single infectious agent.¹ Therapy for drug-susceptible (DS) TB has proven efficient with cure rates of 85–90%.² Success rates of DS-TB treatment are however dampened by the extensive 6- to 9-month therapy duration, accompanying drug toxicity, emergence of drug-resistant (DR) strains and permanent post-TB lung tissue injury.^{2–4} Antimicrobial resistance is an advancing infectious disease threat; resulting in a rise in incidence of drug resistance (DR-TB). Resistance profiles are becoming more complex (i.e more resistant mutants becoming fixed, and onward transmission). The DR-TB treatment regimen pipeline is limited. Proposed regimens use bedaquiline/linezolid/fluroquinolones as backbone drugs. However, resistance to these drugs is emerging (Baseline bedaquiline resistance ~3–5%, baseline fluroquinolones resistance ~15%). Hence, the need for the development of new therapeutic strategies and approaches to improving TB treatment and management outcomes. The global need for new effective therapies has directed a resurgence in efforts to identify new and existing drugs and compounds with potential anti-tuberculous effects. Conventional pathogen-targeted approaches are hampered by the development of emerging microbial resistance. To circumvent this problem, a paradigm shift in drug discovery comprising therapeutic modulation of host cell responses to improve pathogen eradication has emerged.^{5–8} Host-directed therapy (HDT) is unlikely to foster microbial resistance since it targets host cell functions.⁶

Effective host response is a crucial factor for the control and containment of *Mtb* growth. The success of *Mtb* in infecting host cells and maintaining long-term persistent infection is associated with the ability of bacilli to evade host innate as well as adaptive immune mechanisms.^{9–11} HDT is an emerging strategy, where bacterial infection is controlled primarily by limiting *Mtb* growth and restoring the impaired host immune response. We recently published a scoping review on metformin (MET), an FDA-approved drug used to treat type 2 diabetes mellitus and a potential candidate for TB-HDT.¹² Several conclusions have emerged from experimental and retrospective clinical studies, suggesting improved TB treatment outcomes with concurrent MET use. Our review concluded that MET-HDT has the potential to shorten TB treatment and improve treatment outcomes with a possible reduction in TB transmission.

TB disease progression is influenced by *Mtb*-host interactions, where macrophages harbor majority of *Mtb* and have the effector functions to kill these bacilli.^{8,13} Elimination of *Mtb* by macrophage effector functions, halts disease progression and can hasten cure. In contrast, ineffective macrophage activity may result in persistent infection and disease progression. Thus, modulation of macrophage antimicrobial activities may have a significant contribution to containment and elimination of *Mtb* infection. To further delineate the effect of MET as adjunctive TB therapy, this study aimed to determine the effect of MET on the ability of macrophages to control intracellular *Mtb*.

Materials and Methods

Ethics Approval and Institutional Permissions

Blood for peripheral blood mononuclear cells (PBMC) was obtained from HIV-negative donors without TB symptoms who represented healthy subjects with negative TB symptom screening. Written informed consent was obtained from each participant, and the study protocol was approved by the University of KwaZulu-Natal Institutional Review Board (approval BE083/18).

Macrophage Cultures

Peripheral blood mononuclear cells were isolated by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St Louis, MO). CD14⁺ monocytes were purified under positive selection using anti-CD14 microbeads (Miltenyi Biotec, San Diego, CA). A 2 mL of 105/mL monocytes were added to 0.01% fibronectin (Sigma-Aldrich) coated 35 mm glass bottom optical dishes (Mattek, Ashland, MA) and differentiated in macrophage growth medium containing 1% each of HEPES, sodium pyruvate, L-glutamine, and non-essential amino acids, 10% human AB serum (Sigma-Aldrich), and 50 ng/mL GM-CSF (Peprotech, Rocky Hill, NJ) in RPMI. The cell culture medium was changed one day post plating and half the media was replaced on day 3 and 6 post-plating.

Mtb Cultures

The mCherry fluorescent strain of H37Rv *Mtb* was derived by transforming the parental strain with a plasmid with mCherry under the smyc' promoter (gift from D. Russell). *Mtb* were maintained in Difco Middlebrook 7H9 medium enriched with oleic acid-albumin-dextrose catalase supplement (BD, Sparks, MD). Three days before macrophage infection, *Mtb* were switched to grow in Tween 80-free media. On the day of infection, exponentially growing bacterial culture was pelleted at 2000 × g for 10 min, washed twice with 10 mL PBS, and large aggregates broken up by shaking with sterilized 2–4 mm glass beads for 30s. A 10 mL of PBS was added and large clumps were further excluded by allowing them to settle for 5 min. An inoculum of 1 mL was taken from the center of the suspension for macrophage infections.

Mtb Infection

Monocyte derived macrophages (MDMs) were inoculated with 300 ul *Mtb* (*Mtb* dilutions; 1:10, 1:100, 1:1000). After a 3-hour infection and two washes, approximately 5 *Mtb* per MDM or ~300–1000 *Mtb* bacilli per field were observed under a 10x scope. Infected cells were either imaged after 2 hours to allow the *Mtb* to settle, or incubated 18 hours, wash five times to remove extracellular bacteria and then imaged.¹⁴ The method of inoculation and incubation was validated by Mahamed et al. Intracellular growth of *Mycobacterium tuberculosis* after macrophage cell death leads to serial killing of

host cells. *eLife*, 2017. 6: p. e22028. Mahamed et al optimized and validated this protocol in-house which supported the result of TB infection following an 18-hour incubation.¹⁴

Treatment Conditions

The treatment conditions included an untreated control (only infected with Mtb), MET (2mM), INH (25nM) and MET combined with INH. The initial dose of MET was decided based on prior studies^{4,14-17} and a dose-dependent analysis was performed.

Time-Lapse Microscopy

Macrophages and bacteria were imaged using an Andor integrated (Andor, Belfast, UK) Metamorph controlled (Molecular Devices, Sunnyvale, CA) Nikon TiE motorized microscope (Nikon Corporation, Tokyo, Japan) with a 20x, 0.75 NA phase objective. For Mtb RFP and mCherry fluorescence, excitation source was a 561-laser line and emission was detected through a Semrock Brightline 607 nm filter (Semrock, Rochester, NY). Images were captured using an 888 EMCCD camera (Andor). Temperature (37°C), humidity and CO₂ (5%) were controlled using an environmental chamber (OKO Labs, Naples, Italy). Approximately 40 fields of view were captured every 10 min, one phase contrast image and one fluorescent image per field at every time point. Fluorescence readings after death were confirmed by widefield microscopy, but fluorescence readings before and particularly at the point of cell death were strongly influenced by cell movement in the z-plane. For imaging data after cell death, the fluorescence signal starting 4 hours after the cell death event was used for analysis.¹⁸

Determination of Cell Borders and Conversion of Mtb Fluorescence to Bacterial Number

To determine cell borders and conversion of Mtb, phase contrast images were segmented by a custom code using the MATLAB R2018a image analysis toolbox ([Supplementary Material](#)).

Validation of Mtb Fluorescence by Colony-Forming Unit (CFU) Assay Quantitation

To confirm that fluorescence measurements by microscopy reflected actual Mtb expansion, we grew fluorescently labeled-Mtb as a suspension in 7H9 media and sampled aliquots of the suspension by imaging or plating on 7H10 agar plates over a three-day period.

Cytokine and Chemokine Profiles

The protocol used for the assay is described in the [Supplementary Material](#).

Phagocytosis Assay

The protocol used for the assay is described in the [Supplementary Material](#).

Statistical Analysis

Experimental procedures were performed in triplicate (each replicate represents a single donor). p-values of differences between death frequencies between two samples x and y, with $\text{Freq}(\text{death})_x > \text{Freq}(\text{death})_y$, were determined using MATLAB 2018a by randomly selecting with replacement the same number of cells from x, one at a time, as contained in y. The frequency of death in this randomly selected set of cells was determined, and the procedure was repeated 10,000 or 100,000 times to create vector Frand. The p-value was calculated as the number of elements in Frand smaller than $\text{Freq}(\text{death})_y$, divided by the number of times the procedure was repeated. In the case of multiple comparisons, the significance threshold was made more stringent by adjusting for the number of comparisons by the Bonferroni method (Noble, 2009). Statistical analysis was performed using GraphPad Prism 8.

Results

MET Controls Mycobacterial Growth in Live MDMs

We infected human MDMs with the virulent *Mtb* H37Rv strain and measured the ability of MET to control intracellular *Mtb* growth, using a CFU assay over 3 days. In the first 24 hours, treatment with MET controlled *Mtb* growth, and sustained *Mtb* control over 48 and 72 hours (Figure 1A) in live MDMs, similar to INH (Figure 1A). At 72 hours MET *Mtb* CFU count was 15-fold lower compared to the control (Figure 1A). To examine if the observed *Mtb* suppression by MET was host-dependent, we measured *Mtb* growth in the absence of a host cell (Figure 1D). Here, MET had minimal effect on *Mtb* control in the absence of a host cell, suggesting that MET restricts *Mtb* growth via effects on host cell interactions with minimal direct effects on the organism.

To examine if *Mtb* growth was a valid measure of *Mtb* number observed in CFU quantification, we used live cell imaging to a maximum of 72 hours to track infection outcome in individual macrophages (Supplementary Figure S1). MET caused a 14.2-fold decrease in *Mtb* growth compared to the untreated control (Figure 1B). MET combined with INH controlled *Mtb* growth slightly better than INH alone (Figure 1B). MET control of *Mtb* growth observed in CFU quantification correlates with *Mtb* fluorescence signal (Figure 1A–E). We then examined if the observed control in *Mtb* growth influenced MDM survival. Compared to the control, macrophages treated with MET alone demonstrated a 2-fold decrease in MDM death (Figure 1C), INH generated a 5-fold decrease in MDM death (Figure 1C), while MET combined with INH generated a 12.5-fold decrease in MDM death (Figure 1C). We conclude that reduced *Mtb* growth correlated with increased survival of MDMs, following the statistically significant association between decreased *Mtb* CFU, bacterial fluorescence, and number of dead cells.

Potential Synergistic Effect of MET When Combined with Conventional Anti-TB Drug INH

INH, an important first-line agent in TB therapy was used as a comparator to MET. This enabled us to assess INH under the same conditions as MET, and in combination with MET. CFU quantification revealed that INH sustained *Mtb* control

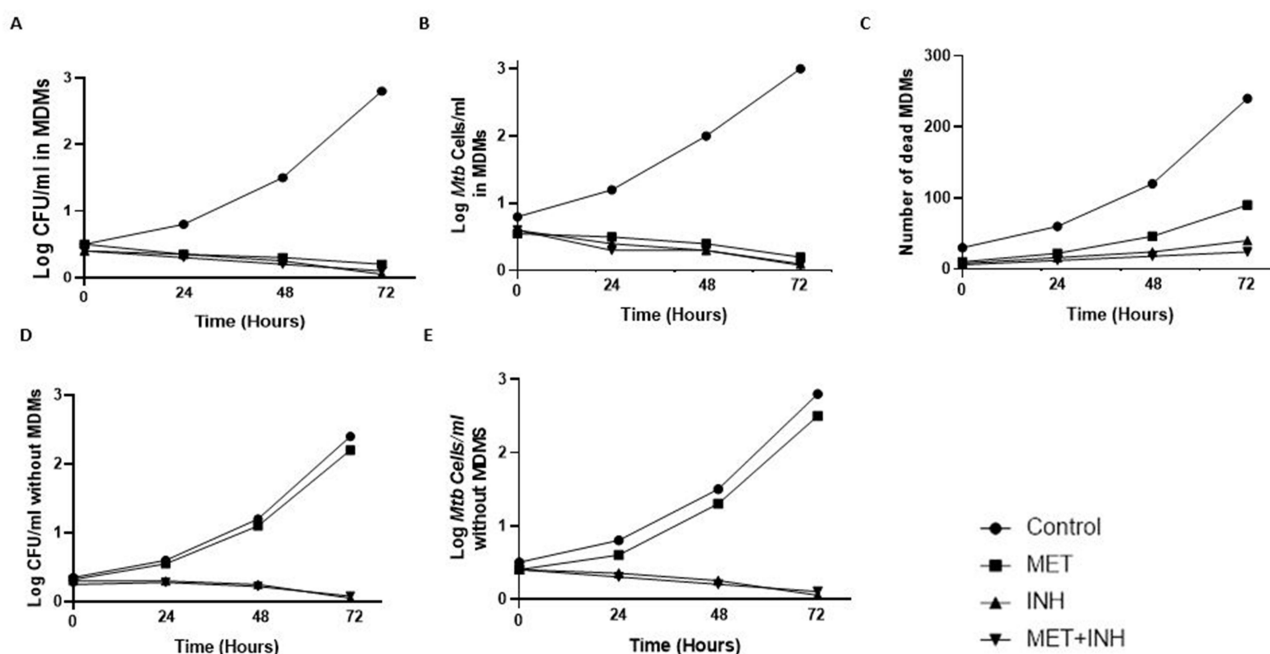


Figure 1 (A and D) CFU quantification of mycobacterial growth. (A) Reduced *Mtb* growth within MDMs treated with MET ($p=0.0017^*$), INH ($p=0.0028^*$) and combination of MET with INH ($p=0.0012^*$). (D) Increased *Mtb* growth without MDMs treated with MET ($p=0.0024^*$), reduced *Mtb* growth without MDMs treated with INH ($p=0.0029$) and combination of MET and INH ($p=0.0015^*$). (B, C and E) Fluorescence signal measured in Cells/mL and log transformed. *Mtb* infections of human macrophages were imaged by time-lapse microscopy at a resolution of 10 min between image acquisitions. Macrophage borders and locations were tracked using a custom-written, MATLAB based image analysis code (Supplementary Material). Graphs B, (C and E) show the fluorescence signal on the y-axis converted to log of the bacterial numbers in the analyzed macrophage and time is hours on the x-axis. (B) Reduced mycobacterial fluorescence signal when treated with MET ($p<0.01221$), INH ($p<0.0311$) and combined MET with INH ($p<0.0131$). (C) Reduction in number of dead cells treated with MET ($p<0.00231$), INH ($p<0.0132$) and combination of MET and INH ($p<0.0133$). Non-parametric, multiple hypothesis, Mann–Whitney *U*-test.

over 72-hours (Figure 1A). Regardless of the presence or absence of a live MDM, INH was able to maintain *Mtb* control (Figure 1A). MET combined with INH exhibited better control over *Mtb* growth. Interestingly, we also observed sustained *Mtb* control even in the absence of MDMs with use of MET combined with INH (Figure 1D). This phenomenon suggests a direct synergistic effect of MET on INH in mediating *Mtb* control. The additive effect of MET on INH, independent of a live MDM, suggests an alternate mechanism for MET to augment *Mtb* control through INH, and is worthy of further evaluation. This potential synergistic effect was validated by live cell imaging. MET with INH significantly limited *Mtb* growth compared to control and INH only (Figure 1). *Mtb* fluorescence signal diminishes in MDMs indicating reduced live *Mtb*. INH caused a 4.2-fold decrease in MDM death compared to the control but not as effective as the 12.5-fold decrease observed by MET combined with INH (Figure 1C). Yet again, we observed correlation between the observed measures – decreased bacterial fluorescence with reduced bacillary numbers and MDM survival, validating the observed synergistic effect of MET combined with INH.

MET Regulates the Pro-Inflammatory Response to *Mtb* Infection

To significantly improve current TB therapy, HDT strategies are required to regulate the inflammatory response, while enhancing anti-*Mtb* defense mechanisms. Release of pro-inflammatory cytokines occurs in response to *Mtb* interaction with macrophages, including TNF- α , IL-1 α/β , IL-6, IL-18, IL-8 and IFN- α/β and chemokines: CCL4, CCL5, CCL20, ICAM-1, TREM-1, GRO- α and GRO- β .^{4,15–21} Under control conditions, there was no change observed in cytokine and chemokine responses from exposure to 72 hours. Compared to control, the following pro-inflammatory cytokine and chemokine expression from *Mtb* infected macrophages was greatest in the first 24 hours when treated with MET: IL-18 (2.3-fold), IL-1 α (2.2-fold), IL-1 β (1.9-fold), IL-8 (1.6-fold), IFN- α (1.6-fold), CCL4 (1.6-fold), CCL5 (1.6-fold), TNF- α (1.5-fold), IL-6 (1.5-fold), CCL20 (1.5-fold), ICAM-1 (1.5-fold) (Figure 2). A similar increase was observed by MET combined with INH: GRO- β (2-fold), IL-1 α (2-fold), IL-18 (2-fold), IL-1 β (1.7-fold), TREM-1 (1.6-fold), GRO- α (1.6-fold), TNF- α (1.4-fold), IL-8 (1.4-fold), IFN- α (1.4-fold), CCL4 (1.4-fold), CCL5 (1.4-fold), ICAM-1 (1.3-fold), IL-6 (1.25-fold) and CCL20 (1.1-fold) (Figure 2).

(Figure 3A–C, Supplementary Figures S2 and S3). We observed a progressive decrease in IL-8 (2.1-fold), IL-6 (2-fold), IFN- α (2-fold), CCL20 (2-fold), IL-1 α (1.5-fold), TREM-1 (1.5-fold), CCL5 (1.5-fold), CCL4 (1.4-fold), ICAM-1 (1.3-fold), GRO- α (1.3-fold), IL-1 β (1.3-fold), TNF- α (1.2-fold), and GRO- β (1.2-fold) and IL-18 (1.1-fold) over 72 hours. These levels were highest in the first 24 hours, subsiding over 48 to 72 hours to reach control levels (Figure 2A–C, Supplementary Figures S2 and S3). Our observation of a significant rise in pro-inflammatory cytokines and chemokines at 24 hours and subsequent decrease by 72 hours in MET treated *Mtb* infected macrophages, amplified with co-administration of MET and INH, suggests that MET independently modulates the host inflammatory response to *Mtb* and potentiates the host inflammatory response induced by INH alone.

Inflammatory Response to Anti-TB Drug INH

We observed a dampening effect of INH on investigated cytokines and chemokines. In the first 24 hours, INH displayed the greatest decrease in pro-inflammatory cytokines: TNF- α (1.7-fold), IL-1 α (1.5-fold), IL-1 β (1.4-fold), IL-6 (1.7-fold), IL-18 (1.6-fold), IL-8 (2.1-fold), IFN- α (1.1-fold), CCL4 (2-fold), CCL5 (3-fold), CCL20 (1.7-fold), ICAM-1 (3-fold), TREM-1 (1.7-fold), GRO- α (1.9-fold) and GRO- β (1.8-fold) compared to the control (Figure 2). This decrease in cytokine levels was observed over 48 and 72 hours. This effect of INH provides evidence on the immune-impairing effects of antibiotics within TB therapy. Conversely, MET combined with INH caused an increase in this pro-inflammatory response. This observed INH associated decrease in pro-inflammatory cytokines is triggered by a drop in bacillary load, which is now overcome by MET. We demonstrated increased bacterial clearance using the phagocytosis assay (Figures 3 and 4) which allowed us to measure phagocytosis within the macrophages. We observed MET to increase phagocytosis 10-fold and MET combined with INH 8-fold compared to the control and INH. MET increased *Mtb* phagocytosis and *Mtb* killing in MET treated macrophages, confirming independent direct effect of MET in *Mtb* clearance.

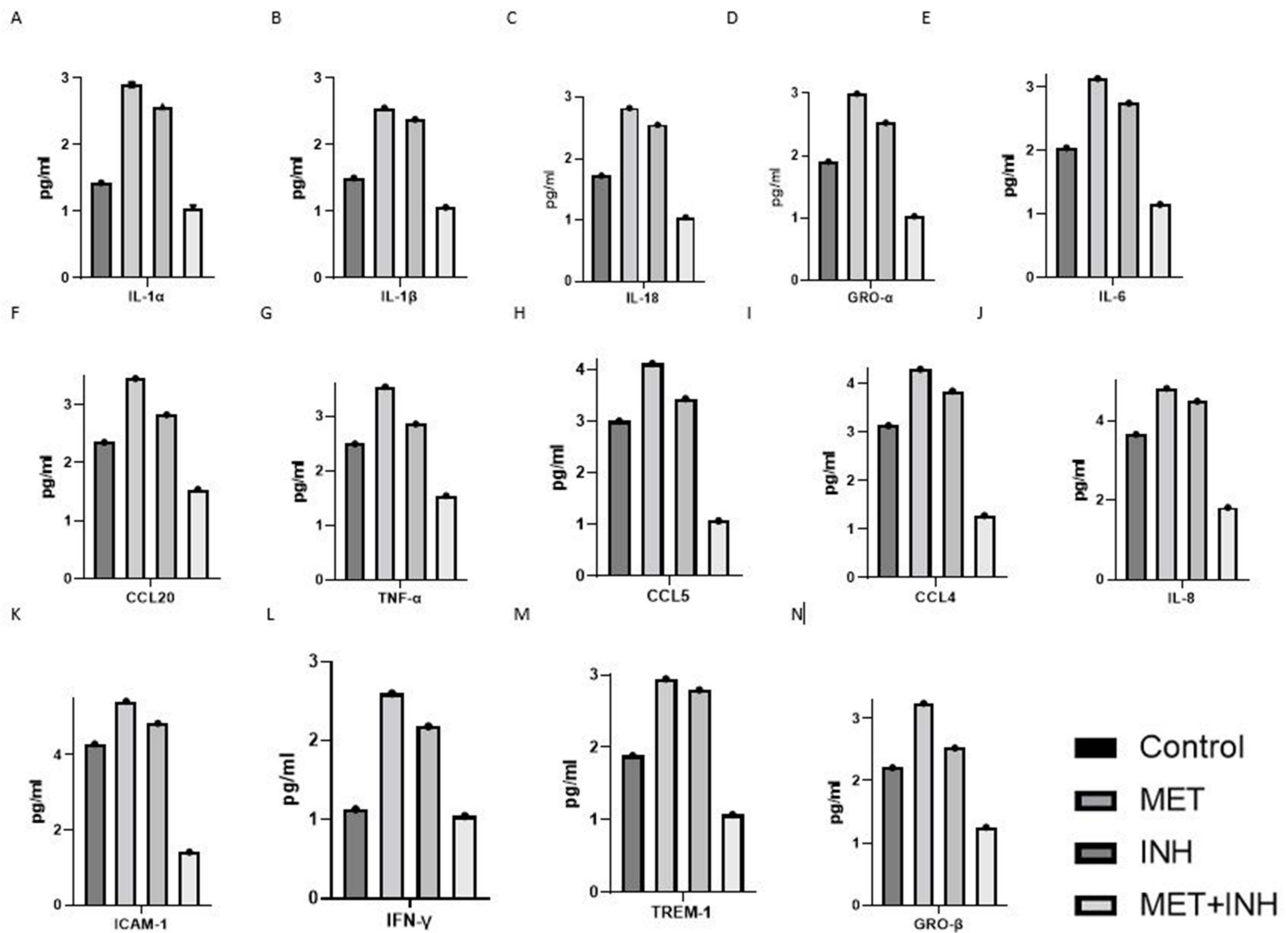


Figure 2 (A–N) MET enhances immune response to Mtb at 24 hours. Mtb infected macrophages were treated with MET, INH and a combination of MET with INH. Evaluated cytokine and chemokine levels: (A) IL-1 α , (B) IL-1 β , (C) IL-18, (D) GRO- α , (E) IL-6, (F) GRO- β , (G) CCL20, (H) TNF α , (I) CCL5, (J) CCL4, (K) IL-8, (L) ICAM-1, (M) IFN γ , (N) TREM-1 (pg/mL). Non-parametric, multiple hypothesis, Mann–Whitney *U*-test. P-values <0.05 were considered significant. MET only and MET in combination with INH increased the phagocytic activity compared to the control and INH. Non-parametric, multiple hypothesis, Mann–Whitney *U*-test. P-values <0.05 were considered significant.

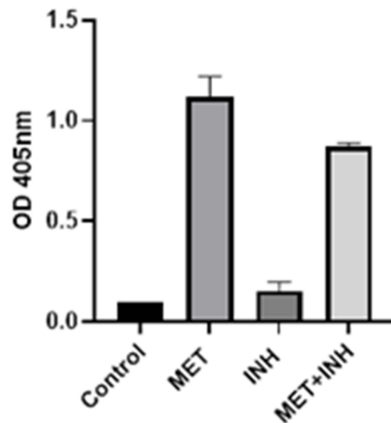


Figure 3 MET enhances phagocytosis within Mtb infected macrophages. 5 \times 10⁴ Raw 264.7 cells/well were seeded overnight in a 96-well plate. MET, INH and MET with INH was used to pretreat MDM cells for 1 hour at 37°C before addition of Zymosan particles at 50:1 ratio. Phagocytosis was stopped after 30 minutes and the amount of engulfed Zymosan particles was determined as described in the assay protocol.

Discussion

The results from our experiments supplement current knowledge on the adjunctive use of MET as HDT for TB (Figure 4). We provide novel data that advances knowledge on mechanisms of Mtb control mediated by MET alone

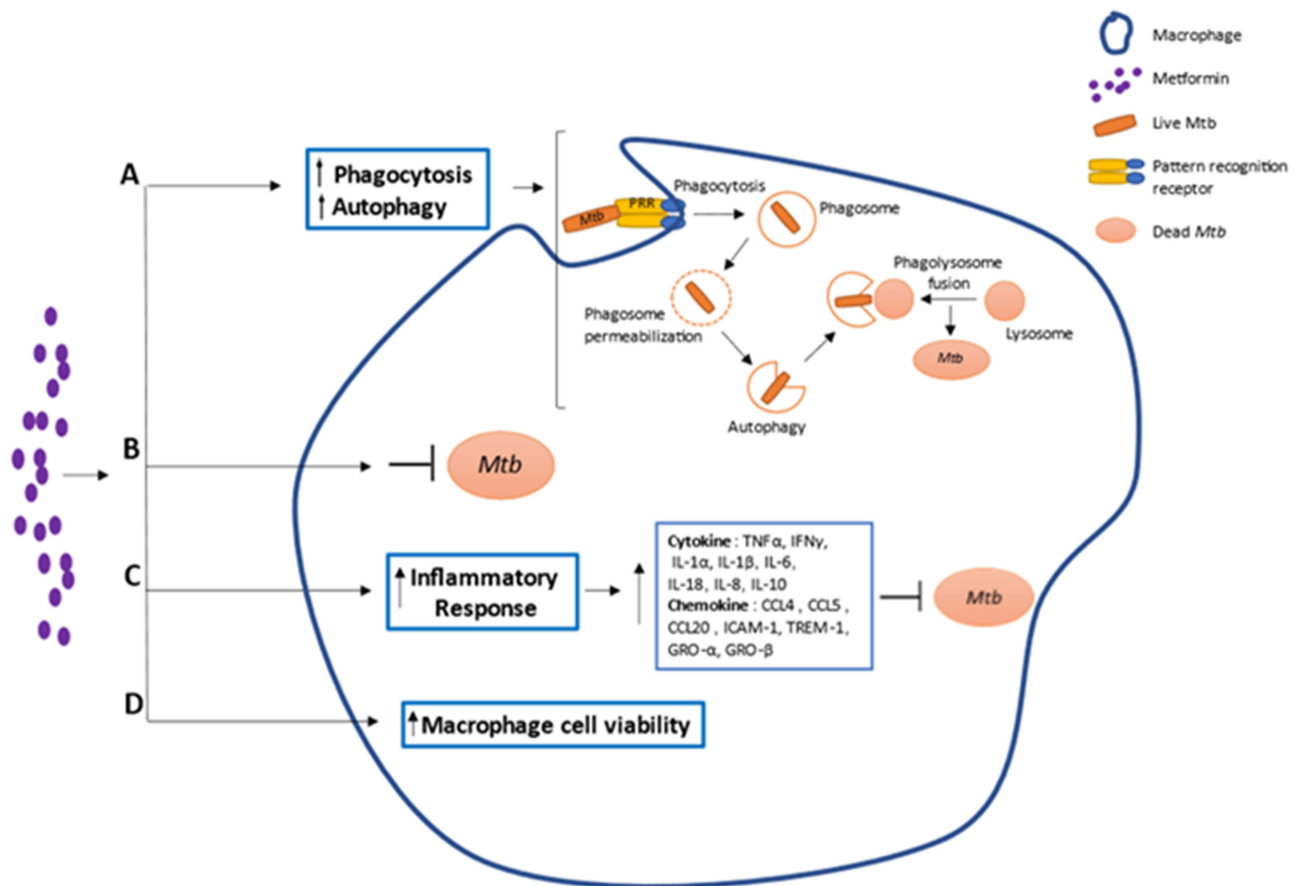


Figure 4 (A) Increased bacterial clearance: We demonstrated MET increases phagocytosis consequently increasing the downstream process of autophagy. MET increased *Mtb* phagocytosis and *Mtb* killing in MET treated macrophages, confirming independent direct effect of MET in *Mtb* clearance. (B) Inhibition in *Mtb* growth: direct effect of MET on *Mtb* growth observed using Time-Lapse microscopy. MET treated macrophages showed 5-fold lower *Mtb* growth than controls. (C) Increased macrophage activation: Demonstrated increased expression of $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$ in MET treated macrophages exhibiting a direct effect of MET on increasing the inflammatory responses to *Mtb* infection. MET treated live macrophages expressed upregulated intracellular pro-inflammatory cytokine and chemokine levels correlating with reduced *Mtb* growth. (D) Macrophage cell viability: time-lapse microscopy was used to determine cell viability. In MET treated macrophages cell viability increased 3-fold compared to INH and control. While previous studies inferred the role of MET on biologic pathways through measurement of pathway by-products such as MAP1LC3B,²² ROS²³ and the ERK1/2-Egr-1 pathway in human monocytes, this study investigated specific biologic pathways and demonstrated direct effect of MET on cellular processes. Our data coupled with published literature advances the current knowledge on the mechanism of MET in mediating *Mtb* killing via a live host cell and controlled pro-inflammatory response.

and in combination with INH. We also provide data demonstrating MET's ability to potentiate the pro-inflammatory response of INH. In addition, we validate pre-existing published evidence on known mechanisms of action of MET. The established pitfalls of traditional antibiotic therapy for TB is evident in the sustained global increase in DR-TB prevalence. This underscores the World Health Organization recommendation for fast-tracking studies evaluating adjunctive TB treatment¹⁵ such as HDT. HDT may potentially augment TB treatment success through limiting *Mtb* growth and modulation of the host immune response, while avoiding development of resistance to anti-TB treatment. Our analysis provides empiric biological data that supports advancement in development of MET as a potential HDT for TB.

Published studies suggest that MET can potentially attenuate bacterial proliferation via unspecified host cell interaction.¹² This view has been incomplete due to lack of insight into dynamic interactions between host immune cells and *Mtb* bacilli. We used an innovative technique of long duration time-lapse microscopy combined with automated image analysis to understand host-pathogen interaction in the presence of MET with and without and INH. Mahamed et al, 2017 successfully used this technique, which provided a validated method to decipher *Mtb* infection dynamics of primary human macrophages.¹⁴ Similarly, live cell imaging enabled us to track *Mtb* infection outcomes of individual primary human macrophages treated with MET and INH. MET when used alone and in combination with INH, mediated a delay in host cell death. This delay allowed for internalization, growth inhibition and control of *Mtb*. Our study

provides empiric data demonstrating that MET alone mediated and maintained a reduction in Mtb colonies within live host cells, confirming MET's effect on the ability of macrophages to control intracellular Mtb. These findings confirm similar observations of reduced 24-hour Mtb survival among MET treated macrophages by Singhal et al,⁴ and adds new knowledge that the effect of MET on macrophage Mtb growth inhibition is sustained for up to 72 hours. We then validated our CFU quantification data with live cell imaging to track bacterial fluorescence and cell death. We observed MET to control Mtb growth and also observed an increase in number of live macrophages over 72 hours, enhancing internalization of Mtb by the host cell. The delay in host cell death caused by MET contributed to our observed increase in live macrophages. We further assessed the effect of MET on the phagocytic activity of MDMs. We observed MET to increase phagocytosis of the host cell, exhibiting a direct pharmacological effect on macrophage function. Interestingly, in an examination of the impact of MET on autophagy by Singhal et al, MET treatment induced autophagy, but if the process of autophagy was blocked, MET-mediated restriction of mycobacterial growth continued.⁴ While these experiments suggest that mitochondrial reactive oxygen species produced early during MET treatment is a key mechanism through which MET restricts the intracellular mycobacterial growth *in vitro*,⁴ we demonstrate that maintaining cell viability is in fact a key mechanism of action.

As expected, INH efficiently controlled Mtb growth regardless of the presence of a viable host cell. We unexpectedly observed a 4-fold reduction in mycobacterial growth and a 2-fold decrease in the number of dead cells from the combined effect of MET used with INH compared to the INH only. MET in combination with INH exerted the greatest effect on maintaining host cell viability and control of bacillary load. Our data is the first to suggest that maintaining a live host cell is likely a key mechanism by which MET restricts intracellular mycobacterial growth and provides the first evidence of a potential synergistic effect of MET when combined with INH allowing for Mtb control. MET enhanced survival of Mtb infected macrophages is central to the host defense. The dual effect of host immune activation highlights the need for an HDT strategy to alleviate host tissue or cell damage by regulating the inflammatory response while enhancing anti-Mtb defense mechanisms, to improve treatment outcomes. We are the first to demonstrate MET mediated altered expression of cytokines and chemokines, signalling MET's ability to modulate the pro-inflammatory response to Mtb. In the first 24 hours, MET displayed the greatest increase in pro-inflammatory cytokines and chemokines: TNF- α , IL-1 α , IL-1 β , IL-6, IL-18, IL-8, IFN- α , CCL4, CCL5, CCL20, ICAM-1 and TREM-1 compared to the control. However, over the period of 48 to 72 hours, MET caused a decrease in these cytokines and chemokines to almost control levels at 72 hours. The significant rise in pro-inflammatory cytokines and chemokines at 24 hours and subsequent decrease at 72 hours provides evidence that MET enables an inflammatory response and regulates this response over time. We also observed MET combined with INH to cause an increase in the investigated pro-inflammatory cytokines and chemokines in the first 24 hours. Similarly, to MET over 48 to 72 hours, there was a decrease in these cytokine and chemokine levels to almost control levels. However, INH only dampened the cytokine and chemokine response to Mtb infection over the entire experimental period. Past studies have observed decreased cytokine production compared to baseline following INH preventative therapy (IPT).^{4,16–23} A possible explanation for this observed reduction could be due to INH-induced apoptosis, however this data has only been generated in one animal study.¹⁸ Past studies suggest that therapeutic antibiotics influence anti-tubercular immune responses. Our results on the dampening effect of INH are in accordance with these studies. However, a novel finding in our study is the combined effect of MET with INH on the initial and sustained host immune response, suggesting MET to have a potential synergistic effect on INH and potentiate the host inflammatory response induced by INH alone. Pharmacological agents like MET that promote pro-inflammatory activity within Mtb-infected cells and the potential synergistic effect of MET on INH could be of great use in eliminating 'silent bacterial reservoirs' in the host and overcoming the emergence of drug resistance Mtb strains.

Conclusion

We provide critical evidence that advances current knowledge on the biologic mechanism of action of MET in TB therapy. Our data suggests MET exerts its HDT effects via maintaining cell viability; allowing MET to enhance macrophage mediated Mtb killing, reduce bacilli growth, increase phagocytosis and mediate host pro-inflammatory responses. Furthermore, our novel finding on the synergistic effect of MET in potentiating the pro-inflammatory response of INH should be considered to re-evaluate the current standard four-drug TB treatment regimen. INH is a component of

DOTS therapy and employed for the entire treatment duration. Moreover, INH monotherapy is used for treatment of latent TB. The synergistic effect of MET on INH could be of great use to prevent the progression of TB infection from latent to active. Our observations warrant consideration for the addition of immunomodulators such as MET to conventional TB treatment regimens. Data from this study adds to the growing evidence that adjunctive use of host-directed and non-antimicrobial pathogen-directed therapies could enhance TB treatment. Further research investigating the benefit of integrating MET with INH may also improve prophylactic control of Mtb, reduce duration of TB treatment,²³ and reduce acquisition of TB drug resistance.

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Disclosure

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