A systems pharmacology approach towards the design of inhaled formulations of rifampicin and isoniazid for treatment of tuberculosis

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Supplemental Text

Methods

Inhaled Carrier Model: Granuloma Compartment

Once in the agent-based model simulation environment, carriers move by random walk (Fig. 1c) with a time step calculated from an estimated diffusivity of carriers in tissue and mucus and scaled based on carrier size (Stokes-Einstein) [1]. Carriers are phagocytosed by macrophages at a probability that is a function of carrier zeta potential (parabolic function), size (Weibull distribution), and surface ligand density (Poisson distribution) and can reside in the intracellular environment for long times (days to weeks) (Fig. S1b-d) [2–5]. If a macrophage dies intracellular carriers are dispersed to the extracellular environment in the Moore neighborhood. Carriers degrade in both the extra- and intracellular space, which can occur at differing rates [6]. Release of antibiotics from carriers occurs in both the intra- and extracellular space as demonstrated by *in vitro* studies of release kinetics [7–12]. We use a description of carrier release kinetics that models both diffusion of antibiotics through the carrier and degradation of the carrier system itself, with time varying boundary conditions [13–15]. We assume the carriers are spherical, such that the system is symmetric in the polar and azimuth angles. If the rate of diffusion is faster than the rate

of degradation ($D/R^2 >> \delta$) then the carrier release kinetics are diffusion-controlled. If the rate of degradation is faster than the rate of diffusion ($D/R^2 << \delta$) then the carrier release kinetics are degradation-controlled (Fig. 1) [13–15]. We solve the release equations for each carrier using a forward-time-central-space (FTCS) finite difference scheme.

Uncertainty and Sensitivity Analysis

We use Latin hypercube sampling (LHS) to simultaneously vary multiple model parameters and sample the parameter space [16]. Partial rank correlation coefficients (PRCCs) quantify the effects of varying each parameter on non-linear outputs, where a PRCC of -1 represents a perfect negative correlation and a PRCC of +1 represents a perfect positive correlation. PRCCs are differentiated based on a students t-test to indicate significance ($p < 0.05$, $p < 0.001$, $p < 0.0001$). We generate 200 unique parameter sets for a specific dosing frequency (daily, two-weeks) of inhaled formulations of RIF and INH, each of which are replicated four times, yielding 1000 simulations per dosing frequency. Average values of model outputs (e.g. CFU, AUC, etc.) 14 days post-treatment are used to calculate PRCC and p-values. In addition, we identify parameter combinations describing inhaled formulations that lead to equivalent sterilization capabilities with reduced toxicity compared to daily oral doses. We sorted the 200 unique parameter sets by comparing total bacterial load and peripheral AUC to the mean values of daily oral dosing. Using the mean value of each parameter in the identified sets and knowledge of parameter PRCCs we design ideal inhaled formulations to test against oral dosing regimens.

Computational Platform

Our hybrid multi-scale agent-based model (ABM) of infection and treatment is constructed using the C++ programming language, Boost libraries (distributed under the Boost Software License),

FFTw libraries (distributed under GPL), and the Qt framework for visualization (distributed under GPL). The ABM is cross-platform and can be run with or without visualization software. Data manipulation was carried out in MATLAB R2012a (Natick, MA). Plots and statistical tests were created using GraphPad Prism 6 (La Jolla, CA).

Model Analysis

Our work investigates antibiotic efficacy at the single granuloma scale. We first simulate 100 days post-infection, whereby a single macrophage is initially infected and a granuloma emerges by ~4 weeks post-infection. Any granuloma that sterilizes before the onset of treatment at 100 days post-infection is removed from analysis. We subsequently treat with antibiotics for an additional 200 days via the inhaled or oral route at two dosing frequencies: daily or every two-weeks. We track average concentrations of antibiotics over time in granulomas, along with concentrations in PK compartments. The peripheral compartment represents organs such as the liver and kidneys, where high concentrations of antibiotics are correlated with increased toxicity [17]. We evaluate hazard ratios (HR) to determine the cumulative risk between inhaled and oral treatments. Uncertainty and sensitivity analysis is used to identify inhaled antibiotic model parameters that have significant effects on model outputs related to treatment efficacy [16].

Results

Extended Sensitivity Analysis

For daily dosing of an inhaled formulation of RIF, the antibiotic loading and antibiotic diffusivity in the carrier are strongly negatively correlated with CFU in granulomas and time to

granuloma sterilization while strongly positively correlated with granuloma and peripheral AUC (Table 1), indicating an important role in treatment efficacy. The intra- and extracellular carrier degradation rates (carrier release kinetics) are significantly correlated with both granuloma and peripheral AUC, but with limited effects on CFU and time to sterilization. Clearance of RIF from the peripheral compartment is significantly correlated with reduced granuloma and peripheral AUC, while weakly correlated with increased CFU and time to sterilization (Table 1). Conversely, dosing every two-weeks with an inhaled formulation of RIF the intra- and extracellular carrier degradation rates (carrier release kinetics) are significantly positively correlated with CFU, while antibiotic loading is still strongly negatively correlated with CFU in granulomas and time to granuloma sterilization. The antibiotic diffusivity of RIF in the carrier increases granuloma AUC yet has limited effects on CFU and time to sterilization.

For daily dosing of an inhaled formulation of INH the antibiotic loading and antibiotic diffusivity (carrier release kinetics) in the carrier are strongly negatively correlated with CFU in granulomas and time to granuloma sterilization, while strongly positively correlated with granuloma and peripheral AUC (Table 2) indicating an important role in treatment efficacy. The intracellular carrier degradation rate (carrier release kinetics) is strongly correlated with granuloma and peripheral AUC, CFU, and time to sterilization, while the extracellular carrier degradation rate (carrier release kinetics) is correlated only with granuloma and peripheral AUC. Clearance of INH from the peripheral compartment is strongly correlated with reduced granuloma and peripheral AUC and increased CFU and time to sterilization (Table 2). Dosing every two-weeks with an inhaled formulation of INH demonstrates that antibiotic diffusivity of INH in the carrier and intracellular carrier degradation rate (carrier release kinetics) are positively correlated with

CFU, while antibiotic loading is again significantly negatively correlated with reduced CFU in granulomas and time to granuloma sterilization.

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