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A model of isoniazid treatment of tuberculosis

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HIGHLIGHTS

- Cell wall thickness affects intracellular drug concentration.
- Intracellular isoniazid creates a feed-back loop via cell wall synthesis.
- Treatment with isoniazid can cause oscillations in cell wall thickness.
- Due to fluctuations in cell wall thickness, cell death may not be monotonic.

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ABSTRACT

A mathematical model is presented of the growth and death of bacilli in a granuloma. The granuloma is treated with isoniazid (INH), a drug that inhibits the synthesis of mycolic acids (MA). Since MA is an essential component of cell walls, the organisms fail to reach maturity if deficient in MA. Cell wall turnover is a well-known feature of bacteria, at the exterior surface material sloughs off to foil attacks by hosts or other organisms, simultaneously synthesizing products for new cell wall assembly. Thus cell wall thickness is maintained in a dynamic equilibrium (Doyle *et al.*, 1988). Presumably cell death is a result of loss in cell wall due to autolysis in combination with stinted replenishing. The mathematical model presented here uses differential equations to predict the effects of intracellular INH on cell wall thickness and cell viability. This analysis purposely distinguishes intracellular INH concentration from the concentration in the plasma. The concentration in the plasma depends only on the dosing. The intracellular INH concentration, however, depends on diffusion through the cell walls of the bacteria. This paper addresses the complex interactions between intracellular INH, cell wall thickness, and the rate of cell wall synthesis.

1. Introduction

Bacteria regulate their cell wall thickness by maintaining a dynamic equilibrium between cell wall lysis and synthesis. The mechanism whereby many antibiotics work is to interfere with proper cell wall maintenance. This is the case with isoniazid, which inhibits the synthesis of an essential component of cell walls in *Mycobacterium tuberculosis* and will be modeled below. Bacteria can counter the antibiotic actions with a range of measures, from efflux pumps to the sophisticated response of *Staphylococcus aureus* to vancomycin (see Salyers and Whitt, 2002; Appelbaum, 2006 for more details). Since passive transport into and out of the cell depends on cell wall thickness, these antibiotics can cause interesting dynamic effects. An example is minimum inhibitory concentration (MIC) study that led to the 'Eagle effect' (or paradoxical killing). It refers to a resistant phenotype which describes a population that shows increased growth at higher concentrations of an antimicrobial agent. The 'Eagle-effect' is a variation on the heterogeneous resistance where the cell population first decreases at the first step, then stabilizes and increases before the final decrease at the second step. This paradoxical phenomenon, where increased concentration of an antibiotic is less effective in eliminating an organism than lower concentrations, was the first observed in several strains of streptococci and staphylococci (Eagle, 1948; Eagle and Musselman, 1948). Since then the so-called 'Eagle-effect' has been observed in a variety of species (Fleischhacker *et al.*, 2008; Grandiere-Perez, *et al.*, 2005). The β-lactam antibiotics also inhibit proper cell wall synthesis and continued autolysis causes cell wall thinning. Studies with *S.aureus* exhibiting resistance to cell-wall targeting glycopeptides (Hanaki *et al.*, 1998; Nunes *et al.*, 2006; Cui *et al.*, 2003) and β-lactams (Morikawa *et al.*, 2001), have shown that the average cell wall thickness has increased. All three of the abovementioned resistance

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Nomenclature

$A(\mu m^2)$ average cell surface
$D(t)$ $(\mu g/ml)$ extracellular INH concentration
D_i $((\mu g/ml)$ INH concentration in bacillus cytoplasm
$F(x,t)$ $(cells/ml)$ cell density
$f(x,t)$ dimensionless cell density
k_L (nm/min) autolysis rate of TB cell wall
$k_G(1/min)$ cell growth rate
$k_D(1/min)$ cell death rate
k_m $(1/min)$ metabolization rate of INH in *M. tb.*
k_s rate of MA synthesis in TB cell
$k_u(\mu m/min)$ rate at which INH is taken up by TB cells
$k_E(\mu m/min)$ efflux rate of INH fromTB cells
$k_{WS}(1/min)$ rate of inserting cytoplasmic MA into cell wall
$M(mycolic\ acid\ molecules/\mu m^3)$ number density of mycolic acid in *M.tb.* cytoplasm
$M_{phys}(mycolic\ acid\ molecules/\mu m^3)$ MA density of in *M.tb.* cytoplasm with INH absent
m dimensionless MA density in *M.tb.* cytoplasm
$t(min)$ time
$V(nm/min)$ rate of change in cell wall thickness
$v(\mu m^3)$ average cytoplasmic volume of TB cell
$x(nm)$ measure of cell wall thickness
$x_l(nm)$ lower viability bound on cell wall thickness
$x_u(nm)$ upper viability bound on cell wall thickness
$x_{Phys}(nm)$ average cell wall thickness in absence of INH

Greek letters

$\alpha(1/min)$ parameter in Eq. (10)
$\beta(ml/\mu g)$ parameter of INH inhibition of MA synthesis
$\phi(1/\mu m)$ defined as $\varphi = \rho_{MA}A/(1000vM_{Phys})$
$\boldsymbol{\rho}_{MA}(mycolic\ acid\ molecules/\mu m^3)$ number density of MA in cell wall

phenotypes (homogeneous, heterogeneous and 'Eagle-effect' resistance) have been observed in *S.aureus* in response to exposure to methicillin (Kondo *et al.*, 2001). The propensity of resistant strains of *S. aureus* to have thicker cell walls than the sensitive strains could be the result of up-regulation of cell wall synthesis (Cui *et al.*, 2003), or down-regulation of autolysis (Gustafson and Wilkinson, 1989).

Returning the discussion to *Mycobacterium tuberculosis*, the results of Velayati *et al.*, 2009 are very interesting. They studied the cell wall structures of sensitive TB, multi-drug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains by TEM analysis. The thickness varied from 15.6 ± 1.3 nm for the susceptible isolates to 17.1 ± 1.03 nm and 20.2 ± 1.5 nm for MDR and XDR-TB bacilli respectively. Even thicker walls were found in a small percentage of XDR bacilli. The observation by Lemmer (2010) provided the major idea to this study. PLGA nanoparticles with INH and mycolic acid payloads (to better target infected macrophages) provided poorer coverage than PLGA nanoparticles with INH alone. The author posited that "The inclusion of MA into the nanoparticles seemed harmless to the host macrophages, but decreased the mycobactericidal efficiency of INH somewhat, an observation that may be attributed to INH inhibiting the natural MA biosynthesis of the pathogen, while inclusion of MA in the NP adds an external source of this metabolite to the pharmacologically stressed *M. tuberculosis*" It is known that MA is not only an essential component of the cell wall, but it plays a causal role in the tolerogenic response of infected alveolar macrophages and the possible recruitment of cholesterol – a putative food source for mycobacteria. The MAs also facilitate lipophilic transport across the cell wall. INH could compromise all these functions. For the purposes of this study, however, the most important consequence of the drug is the inhibition of cell wall synthesis through its effect on MA. Dartois (2014) provided an in-depth look at the complexity of drug delivery to bacilli that are in granulomas, and her work underscores the need for more effective drug delivery systems. Plasma concentrations of anti-TB drugs are not necessarily a good indicator of the concentrations in bacilli.

Pienaar *et al.* (2009) have developed a mathematical model to describe the complex response of *S. aureus* to methicillin, including the 'Eagle effect'. They modeled the cell population as a probability density distribution of cell wall thickness. The Fokker–Planck equation, which describes the movement of the distribution, was solved for different rates of cell wall synthesis, autolysis and antibiotic concentrations. In this study we focus on sensitive TB. All bacilli are initially at the average cell wall thickness associated with drug-free conditions. In addition to the cell population, the MA synthesis and dynamic cell wall thickness are modeled. Also, the plasma and intracellular concentrations of INH are included.

2. Mathematical model

The bacilli are all sensitive to INH at the onset of the simulation and reside within the macrophages of an early vascularized granuloma Dartois (2014) referred to this type of granuloma as a cellular granuloma.) In the later stages of the disease, the model proposed here may not apply because INH has significantly lower effectiveness against non-replicating bacilli (Niki *et al.*, 2012). Initially all bacilli have the same cell wall thickness. The bacilli population $F(x,t)$ is a function of cell wall thickness x and time t. We define an upper and lower limit on cell wall thickness within which cells are viable, namely $x_{u,l}$. The model describes the changes in cell wall thickness as a function of INH and MA. The concentration of mycolic acid in the bacterial cytoplasm will be denoted as M. We define the plasma concentration and the intra-bacterial concentration D and D_i respectively. Since INH is a pro-drug (Metcalfe *et al.*, 2008; Mahapatra *et al.*, 2012) that is converted to its active form in the cytoplasm by *KatG* peroxidase, our model assumes that this activation step is not rate limiting. Experimental support for this assumption can be found in the literature (Bardou *et al.*, 1998). Also define the surface area/cell as $A(\mu m^2)$ and the mycolic acid content of the outer cell wall as $\rho_{MA}(\text{mycolic acid molecules}/\mu m^3)$. Strictly speaking A changes over the course of cell division, but growth occurs primarily at the lateral wall (not at the poles), in other words rod extension, (Hett and Rubin, 2008). Since there is some dispute about rod extension (Aldridge *et al.*, 2012; Thanky *et al.*, 2007), in this study we treat $A(\mu m^2)$ as a time averaged quantity over one cell cycle. At drug-free conditions the cells are at a physiologically ideal state with cell wall thickness $x_{phys} = 15.6$ nm. (Average cell wall thickness for WT strain, Velayati *et al.*, 2009). Let $f = F(x,t)/F(x,0)$, where $F(x,0)$ is the number density of cells (cells/ml) prior to antibiotic treatment.

The governing equation is:

$$\frac{\partial f}{\partial t} + V(D_i, M)\frac{\partial f}{\partial x} = k_G e^{[x_l - x_{Phys}]}\frac{f}{1+f} - k_D e^{[x_l - x]} f \tag{1}$$