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A unified anti-mutant dosing strategy

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Antimicrobial dosing is currently attracting attention as a way to minimize the emergence of resistance. Three dose-based strategies have been advocated, each with shortcomings. Focus on killing susceptible cells overlooks resistant mutant subpopulations that may be present before treatment or generated during therapy; keeping therapeutic drug concentrations above the mutant prevention concentration (MPC; resistant mutant MIC) may be overly stringent; and dosage escalation modelling uses indirect estimates of resistant mutant subpopulation susceptibility (multiples of bulk population susceptibility, MIC) rather than direct estimates from MPC. The latter is significant because MPC and MIC are discordant with multiple pathogen isolates. Combining the strategies leads to MPC-based PK/PD thresholds (e.g. AUC_{24}/MPC and $t >$ MPC) for restricting resistant subpopulation enrichment and amplification. Using MPC-based thresholds to model dosing regimens that will restrict emergence of resistance requires generation of databases in which MPC is determined for many isolates.

Keywords: MPC, PK/PD indices, mutant selection window

Introduction

Antimicrobial resistance continues to make headlines, despite calls for restricting unnecessary antimicrobial use. Highly visible resistance problems, such as community-acquired methicillin-resistant *Staphylococcus aureus*,[1,2] are generally caused by transmission of resistant pathogens from one person to another. Although transmission can often be limited by infection control methods, ultimately, we need to control its precursor, the acquisition of resistance. Several approaches have been proposed. The traditional strategy focuses on killing susceptible cells, as that should reduce the pool from which new mutants arise. More recently, emphasis has shifted to direct control of resistant subpopulations. They can selectively amplify under antimicrobial pressure and eventually dominate a population; at that point, antimicrobials and host defences may fail to control pathogen growth.

Although resistance is often considered an absolute term clinically, growth of many 'resistant' mutants can be inhibited by drug concentrations attainable therapeutically [such isolates have intermediate (dose-dependent) susceptibility]. The distinction between absolute *clinical resistance*, which may be used as an endpoint for modelling studies, and *resistant mutants*, which may be controlled by higher drug concentrations, is important for considering ways to halt the acquisition of resistance. Such considerations can lead to dosage increases[3,4] or shifts from monotherapy to multidrug combination therapy.[5] Below we sketch out three dosing strategies aimed at slowing the emergence of mutational resistance and then consider combining their best features. Induced phenotypic resistance, a situation in which all members of the pathogen population exhibit reduced susceptibility, is outside the present discussion.

Dosing to eradicate susceptible cells

In principle, reducing the bacterial load should suppress the acquisition of new mutants.[6] Killing susceptible cells may also enable host defences to more effectively eliminate residual subpopulations of resistant mutants. Suitable antimicrobial doses are identified by measures of pathogen drug exposure that empirically correlate with the eradication of susceptible bacteria. For antimicrobials that are considered 'time-dependent killers', the pharmacodynamic term is time above MIC ($t >$ MIC); for 'concentration-dependent killers', it is either area under the concentration–time curve in a 24 h period divided by MIC (AUC_{24}/MIC) or maximal drug concentration divided by MIC (C_{max}/MIC).[7]

As drug concentrations that kill susceptible cells may allow mutants to amplify,[8–10] a dosing approach that addresses only bulk population susceptibility, which is measured by MIC, fails to consider resistant mutant subpopulations. Consequently, resistance can emerge during the eradication of susceptible cells (Figure 1), as seen with *S. aureus* colonizing the noses of tuberculosis patients.[11] When a set of these patients was treated with rifampicin for tuberculosis, susceptible colonizing *S. aureus* was

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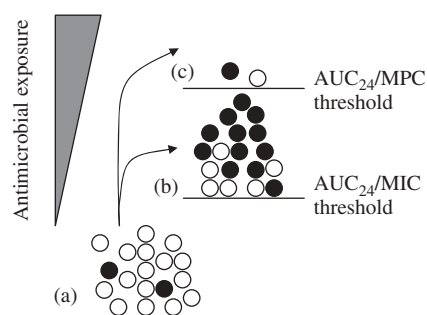
Figure 1. Effect of PK/PD thresholds on major susceptible and minor resistant pathogen populations treated with a lethal agent. (a) Unchallenged bacterial population contains a majority of susceptible cells (open circles) and a small subpopulation of resistant mutants (filled circles). (b) When drug exposure passes an AUC_{24}/MIC threshold above which mutants, but not susceptible cells, can survive and proliferate, enrichment and amplification of resistant mutant subpopulations occur. (c) When drug exposure exceeds an AUC_{24}/MPC threshold above which resistant mutants and susceptible cells are both killed, acquisition of resistance fails to occur (a few pathogen cells may persist, as shown). With fluoroquinolones, AUC_{24}/MPC of 20–70 restricts emergence of resistance.[31]

eradicated, but in 10% of the patients, rifampicin-resistant *S. aureus* was acquired. Thus, treatment strategies aimed only at eradicating susceptible cells may not block the acquisition of resistance.

Drug concentrations above mutant prevention concentration (MPC)

A second approach involves direct measurement of resistant mutant subpopulation susceptibility (MPC).[12] MPC is estimated as the drug concentration that blocks growth when 10^{10} cells are applied to agar[12] or tested in liquid medium[13] (MIC determination uses 10^4–10^5 cells). Large inocula ensure the presence of mutant subpopulations; consequently, MPC estimates resistant subpopulation susceptibility. If antimicrobial concentrations are kept above MPC throughout therapy, mutant subpopulation amplification will be inhibited. How and when resistant subpopulations are generated has no influence on this approach, which holds for both bacteriostatic and bactericidal agents. MPC has been measured for fluoroquinolones, linezolid, macrolides, β-lactams, vancomycin and daptomycin (the major failure to measure MPC has been for rifampicin with *Escherichia coli*).[14–17] The strategy of maintaining drug concentrations above MPC suffers from being more stringent than necessary for antimicrobials that kill *resistant* pathogens. For example, with a local *S. aureus* infection of rabbits, levofloxacin concentrations need to be above MPC for only 20% of the dosing interval to restrict mutant amplification.[8]

Dose escalation modelling

A third approach involves mathematical modelling of data obtained with escalating doses to identify the susceptible population-based drug exposure (AUC_{24}/MIC) that blocks amplification of resistant mutant subpopulations and kills most susceptible cells.[18] Monte Carlo simulation is then used to estimate the fraction of patients for which a given dose will achieve the restrictive value of AUC_{24}/MIC.

A key assumption with this method is that dominant population susceptibility (MIC) is proportional to resistant subpopulation susceptibility (MPC) among many different clinical isolates, thereby allowing particular multiples of MIC to approximate mutant subpopulation susceptibility. Although proportionality is by definition true for any single isolate, experimental measurements indicate that MPC and MIC are discordant when multiple isolates are surveyed.[19–21] Lack of proportionality may arise from patient isolates harbouring mutations that confer little increase in MIC, but a large increase in MPC and vice versa.[22–24] Thus, MIC-based PK/PD thresholds are likely to be inherently less accurate for suppressing selective amplification of resistant mutant subpopulations than those based on direct measurement of resistant subpopulation susceptibility (e.g. AUC_{24}/MPC). The dose escalation modelling also requires testing enough cells to assure that amplification of the least susceptible, single-step mutant is suppressed.[18,25]

Combined approach

As problems with the dose escalation modelling approach derive from using bulk population susceptibility rather than mutant subpopulation susceptibility, an improvement would be to use an MPC-based threshold (for example, AUC_{24}/MPC) rather than an MIC-based one (for example, AUC_{24}/MIC) as a target parameter for blocking resistant subpopulation proliferation. The same conclusion is drawn from a slightly different argument. Empirical relationships between drug exposure and efficacy identify AUC_{24}/MIC for some agents and $t > MIC$ for others, as the PK/PD index that relates to bulk pathogen survival.[7] These indices for susceptible cells should apply to resistant subpopulations if MPC is substituted for MIC, as MPC correlates with the MIC of the least-susceptible single (next)-step mutant.[26] Then, AUC_{24}/MPC and $t > MPC$ would be thresholds for preventing resistant mutant subpopulations from proliferating (see also Olofsson *et al.*[27]). In principle, C_{max} could be substituted for AUC_{24}.[8,28] However, this would require more complex population pharmacokinetic expressions, as AUC_{24} includes a consideration of dosage frequency, which is absent from raw C_{max} measurements.

Conclusions

Halting the selective amplification of resistant mutant subpopulations requires more stringent antimicrobial therapies than required to clear susceptible infections. For several reasons, values of AUC_{24}/MPC and $t > MPC$ are expected to be preferred PK/PD thresholds for defining dosing regimens that block mutant subpopulation proliferation. This expectation must now be confirmed with animal infection models. The application of these expressions to large patient populations requires a standard methodology for the measurement of MPC[16,25,29,30] and then generation of extensive databases of population MPC.

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