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Narrative Review

Assessment and modelling of antibacterial combination regimens

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ABSTRACT

Background: The increasing global prevalence of multidrug-resistant bacteria is forcing clinicians to prescribe combination antibiotic regimens to treat serious infections. Currently, the joint activity of a combination is quantified by comparing the observed and expected effects using a reference model. These reference models make different assumptions and interpretations of synergy. They fail to: (i) account for multiple bacterial subpopulations with differing susceptibilities; (ii) quantify or interpret the explicit interaction (synergy/antagonism) mechanisms; and (iii) accommodate spontaneous mutations.
Aims: To develop better study designs, mathematical models, metrics and pharmacodynamic analyses to assist with the identification of highly active combinations that are translatable to the clinical context to address the mounting antibiotic resistance threat.
Sources: PubMed, references of identified studies and reviews, and personal experience when evidence was lacking.
Content: We reviewed metrics and approaches for quantifying the joint activity of the combination. The first example is using experimental data from an *in vitro* checkerboard synergy panel to develop and illustrate a less model-dependent method for assessing combination regimens. In the second example a pharmacokinetic/pharmacodynamic model was developed using mechanism-based mathematical modelling and monotherapy and combination therapy data obtained from an *in vitro* hollow fibre infection model evaluating linezolid and rifampin regimens against *Mycobacterium tuberculosis*.
Implications: Mechanism-based mathematical approach provides an excellent platform for describing the time course of effect while taking into account the mechanisms of different antibiotics and differing pathogen susceptibilities. This approach allows for the future integration of 'omics' data describing host–pathogen interactions, that will provide a systems-level understanding of the underlying infectious process, and enable the design of effective combination therapies. **G.G. Rao, Clin Microbiol Infect 2018;24:689**

Introduction

The rapid emergence and dissemination of multidrug-resistant (MDR) bacteria represents a major public health issue, not only in terms of disease burden but also through diminished antibiotic efficacy [1–3]. The first global report on antimicrobial resistance by the WHO concluded that antibiotic resistance seriously threatens antibiotic use, with the possibility of a 'post-antibiotic' era leaving us without treatment options [4]. Antibiotic-resistant pathogens are increasingly common, forcing clinicians to resort to prescribing multi-drug regimens to treat serious infections [5–8]. Additionally, the discovery and approval of new antibiotics has declined. This has resulted in increased interest in repurposing existing antibiotics, which represents an essential strategy to combat mounting antibiotic resistance [9].

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The rationale for antibiotic combination therapy is that the traditional 'one drug–one target' approach cannot provide the necessary broad-spectrum activity required to treat infections caused by these resistant pathogens [10–12]. Combination therapy for the treatment of diseases like human immunodeficiency virus (HIV), hepatitis C virus (HCV), malaria and cancer resulting in prolonged survival and transforming the rapid and lethal nature of these diseases [13–15]. The success of combination therapy has prompted researchers to 'borrow' strategies for the design of antibiotic combination therapy. Combination therapy can increase the probability of clinical success by: (i) maximizing the decrease in bacterial load; (ii) minimizing the emergence of resistance; and (iii) decreasing toxicity and drug-related adverse events. Furthermore, we now know from basic and translational studies that the pathobiology of infectious disease involves interconnected molecular pathways containing multiple distinct drug targets susceptible to the simultaneous action of several drugs in combination [12,16–18].

Nevertheless, the use of combination regimens in the clinic is often empirical and not supported by systematic or clinical trial data. Research in the area of combination therapy has generally focused on proving the superiority of combination therapy over monotherapy. Considerable effort has been made to define metrics that define synergistic combinations as opposed to quantifying the joint activity of combinations. Therapeutic success depends on the total activity achieved at the infection site against the infecting pathogen [19]. Synergy or antagonism 'contributes' to activity and is of interest, but a synergistic regimen is not necessarily effective; two drugs with high MICs, maximally dosed, and with substantial synergy may still represent an ineffective regimen. Antagonism is not always a bad thing; for example, vancomycin plus rifampin against methicillin-resistant *Staphylococcus aureus* can be antagonistic but still might be more effective than either drug alone or any other two-drug combination [20]. There are also data to suggest that the presence or absence of synergy *per se* does not help to guide the selection of combinations in the clinical setting [21]. Furthermore, definitions of synergy or antagonism have not been standardized within the pharmacology or infectious diseases [22].

With the increasing global prevalence of MDR strains, it is imperative that we develop better mathematical models, metrics and pharmacodynamic (PD) analyses and study designs that can assist with the identification of highly active combinations that are translatable for use in the clinical context. The ability to quantify interactions between antibiotics is a major challenge when assessing antibiotic combinations. Synergy, additivity and antagonism are major terms that have been used to describe these drug interactions. The effect of the combination is often quantified by comparing the observed effect of the combination with the expected effect using a reference model. When the observed effect of the combination is greater than expected, the combination is classed as 'synergistic', whereas effects that are less than expected are described as 'antagonistic'. Two reference models are widely employed to quantify joint activity, the Bliss independence model [23] and the Loewe additivity model [24], which use different assumptions and interpretations of synergy.

These reference models do not address three main issues unique to infectious diseases. First, they fail to recognize or account for the presence of multiple bacterial subpopulations with different susceptibilities [25]. Second, they fail to quantify or interpret the explicit interaction (synergy/antagonism) mechanisms; and lastly, they cannot accommodate spontaneous mutations. This is becoming more important as most resistant bacterial strains are heteroresistant, with subpopulations differing (at least) in their susceptibilities to the different drugs used in combination.

We have reviewed Bliss Independence and Loewe Additivity models as either of these models are not entirely suitable to model antibiotic combinations but both models have elements that are suitable and have been included in our modelling approaches discussed in the manuscript. Given the limitations of these existing reference models, here we suggest and discuss some of the metrics and PD analyses that help to quantify joint activity and characterize the interaction between antimicrobials in the effort to identify optimal antibiotic drug combinations.

Metrics for quantifying interactions between drugs

Bliss independence uses probabilistic theory to model the combined effect of two antibiotics assuming that neither drug affects the other. Based on the quantitative analysis, the effect of drugs used in combination is equal to the sum of the expected effects, i.e. effects are additive (log domain).

The individual drugs used in combination act as if they have distinct mechanisms of action or targets. The effects of Drug A in combination with Drug B may be modelled using a Hill-type model, in which D_A and D_B are drug concentrations. The Hill equation parameters include: E_0, the baseline effect; E_{maxA} and E_{maxB} (maximal drug effect, potency); EC_{50A} and EC_{50B} (drug concentrations that produce 50% of maximal effect, sensitivity); and H_A and H_B, Hill coefficients describing the steepness of the relationship for antibiotics A and B, respectively. Based on the Bliss independence model, 'the effects of the drugs used in combination are additive' rather than the doses.

$$\textit{Joint Drug Effect} = E_0 - \left(\frac{E_{max_A} \cdot D_A^{H_A}}{E_{50_A}^{H_A} + D_A^{H_A}} + \frac{E_{max_B} \cdot D_A^{H_B}}{E_{50_B}^{H_B} + D_B^{H_B}} \right) \quad (1)$$

The PD of Drug A and Drug B are 'independent', if neither drug enhances nor attenuates the effects of the other drug. Furthermore, Drug A is equally active against bacteria that are sensitive or resistant to Drug B and similarly Drug B is equally active against bacteria that are sensitive or resistant to Drug A. Any or all of the Hill parameters can differ between drugs. A dose of Drug A that yields 2 $\log_{10}$ of net kill, combined with a dose of Drug B that yields 3 $\log_{10}$ of kill would be predicted to yield 5 $\log_{10}$ of kill; the joint E_{max} is assumed to be the sum of the two effects. This predicted effect would indicate that the combination is consistent with independence, whereas a decrease greater than 5 $\log_{10}$ would indicate interaction (synergy, in this case).

In Fig. 1, drug combinations with joint activity in the area below the Bliss independence line are designated synergistic by either reference model. Combinations with joint activity in the region above the Bliss independence line would be regarded as antagonistic by the Bliss independence model. Hence, based on the Bliss independence definition, effective antibiotic combinations that result in a 3 to 5 $\log_{10}$ reduction in the bacterial load will be labelled 'antagonistic' even though the joint effect is more than that possible according to the maximum effect of either drug alone. The pharmacology literature seems to equate Bliss independence with additivity. We do not believe that this is appropriate; the apparent independence of actions is a valid standard, but such independence is not additivity.

By contrast, Loewe additivity assumes that the drugs used in combination act on the same pathway or target through a similar mechanism of action. Loewe additivity assumes 'similar' E_{max}, E_o and H; and drugs differ in their sensitivity. After adjustment for sensitivity (dividing concentrations by EC_{50}), 'doses (concentrations) are additive'. A guideline for Loewe additivity is that the combination of a drug with itself should be classified as additive (by Bliss, this cannot be true; a drug combined with itself would be