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Short communication

Analysis of drug-drug interactions: an overview[1]

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Clearly, the continued discovery of new drugs and the investigation of new targets are both essential for the development of effective antiviral chemotherapy. However, other strategies exist. One attractive approach to this problem is to optimize the efficacy and selectivity of currently existing drugs by combining them with other agents.

To realize the potential benefits of combination therapies, a careful analysis of the interactions between drugs is required. This is essential because it is not possible to predict how drugs will interact in a virus-infected cell. Many drug combinations that synergistically inhibit viral replication also are synergistically toxic and do not improve the selectivity of the drug. Furthermore, some combinations of antiviral drugs may unexpectedly antagonize their respective antiviral activities and result in reduced efficacy.

Many different experimental designs and analytical approaches have been developed for analyzing experimental data. Before examining these methods, it is necessary to briefly address the enigmatic concept of 'expected' effect. Most would agree that synergy is defined as greater than the expected effect and antagonism is defined as less than the expected effect. Many discussions on the definition of synergy have been unable to resolve the issue of defining expected effects. Two major mathematical definitions of expected effects exist; namely, 'Bliss independence' and 'Loewe additivity'. This nomenclature was suggested recently by a group of investigators attempting to reach a consensus on concepts and terminology for combine-action assessment (Greco et al., 1992). Bliss independence is based on statistical probability and assumes that two drugs should act independently to affect virus replication. Loewe additivity is based on dose-addition and assumes that two drugs should be indistinguishable from each other with respect to antiviral effects in a combination. The equations for both Bliss independence and Loewe additivity can be confirmed with experimental models and both have endured frequent criticism.

The interaction of two drugs is a problem of three dimensions (3-D). Three variables exist: two independent variables (the concentrations of the

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two drugs) and a dependent variable (the biological effect of the combination). As a result, these interactions are best described by a 3-D dose-response surface. Historically, 2-D analytical procedures were used to approximate the three-dimensional does-response surface. Today, over twenty 2-D and 3-D analytical methods are available to summarize experimental data and to characterize the interactions between drugs. This topic has been reviewed previously by several authors, the latest offering being by Greco et al. (1995). Most if not all of these analytical methods can be grouped into the six general categories seen in the Table 1. Each of these general categories is described briefly in the following text.

The fractional product category contains 2-D methods that use the Bliss independence equation to predict the expected effects at single points on the dose-response surface. A ratio of the expected effect to the actual effect is used as an indicator of synergy (<1) or antagonism (>1). This approach can accurately define the dose-response surface but the results from many different points have to be calculated and plotted.

Multiple dose-response curves methods, as the name implies, plot a series of dose-response curves for one drug with the addition of fixed concentrations of a second drug. Typically, the expected effects are not calculated and conclusions are simply based on the shift of the dose-response curve.

Isobolograms have been used for many years by different investigators. These plots are equivalent to taking horizontal cross sections through the 3-D dose-response surface. More recent versions of this model allow a statistical analysis of the lines. Traditionally, isobolograms have used Loewe additivity because this equation yields a straight line when the drug doses, or a function of the drug doses, are plotted with a linear scale. 'Expected' isobolograms are not linear when logarithmic scales are used, or when the Bliss independence equation is used.

The combination index method is a widely used and accepted 2-D method for analyzing drug interactions in many fields. This analytical method was designed to use data from experiments with a constant molar ratio. Typical fixed ratio experiments can explore fairly large regions of the dose-response surface. However, fixed ratios follow parallel diagonals across the dose-response surface when log scales are used and can leave large regions untraversed. A recent update of this analytical technique incorporates Monte Carlo simulations to estimate the statistical significance of the combination indices (Belen'kii and Schinazi, 1994).

Three dimensional methods were developed to plot the dose-response surface to identify regions of the surface where synergy or antagonism is present. This type of analysis is easier to interpret than the 2-D analytical methods as it graphically presents the data in the appropriate 3-D context. The method of Prichard and Shipman (1990) calculates expected effects and subtracts this surface from the experimental dose-response surface to reveal statistically significant differences. The resulting plot yields a plane at 0% expected inhibition with synergy represented by a peak above the plane and antagonism as a depression below the plane. Another differential surface analytic method uses spline functions to construct a 3-D dose-response surface (Sühnel, 1990). This method uses the shape of the 3-D surface to characterize drug interactions, much like isobolograms. Like isobolograms, no quantitative measure is given and no statistical tests are performed.

Table 1
General categories of analytical methods used to analyze drug-drug interactions

Analytical method	Approach	Assumptions
Fractional product	2-D	Independence
Multiple dose-response curves	2-D	Independence
Isobologram	2-D	Additivity
Combination index	2-D	Additivity[a]
Differential surface analysis	3-D	Additivity or independence
Parametric surface fitting	3-D	Additivity

[a]In addition to equations for Loewe additivity, another equation that is close to but not equivalent to Bliss independence is available.

The parametric surface fitting approach uses response surface methodology to fit equations to the experimental data (for examples, see Bunow and Weinstein, 1990; Carter et al., 1985; Greco et al., 1990; and Machado and Robinson, 1994). The methods cited here all use the approach of surface fitting, but use different equations and different parameters to fit the experimental data. The resulting mathematical parameters are used to make conclusions about the shape of the dose-response surface. These methods use different parameters as indicators of synergy, antagonism and statistical significance. One common characteristic of these complex models is that they are difficult to use and will generally require consultation with a statistician familiar with the method.

For any given analytical approach, it is imperative that the investigator clearly understand the assumptions of the method used and the implications of the results. The rigorous analyses provided by currently available analytical methods should yield insights regarding the interactions between drugs and provide valuable and, hopefully, predictive preclinical information.

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