II *Letter to the Editor* in response to J. Sühnel's comment on the paper: A three-dimensional model to analyze drug-drug interactions. Prichard, M.N. and Shipman, C., Jr. (1990) Antiviral Res. 14, 181–206.

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As we have stated in our review, significant disagreement exists on the definition of additivity (zero interaction), which was reiterated in the preceding letter. The definition of expected additivity is crucial because synergy is defined as being greater than the expected effect, and antagonism is defined as being less than the expected effect. The current three-dimensional (3-D) analytical methods (Greco et al., 1990; Prichard and Shipman Jr., 1990; and Sühnel, 1990) all calculate additivity differently.

The methods proposed by Sühnel and Greco et al. are based on the work of Berenbaum (1985, 1989). Berenbaum assumed that linear isobolograms always show 'zero interaction' or additivity and proposed 'the general solution' (eqn. 1):

$$\sum_{n=1}^{i} \frac{x_i}{X_i} + \frac{y_i}{Y_i} = 1 \tag{1}$$

In this equation, 'X' is equal to the concentration of drug X that produces an effect and 'Y' is equal to the concentration of drug Y that produces the same effect; 'x' and 'y' are equal to all combinations of concentrations of the first and second drugs, respectively, which in combination produce the same effect as do X and Y individually.

Berenbaum assumed that all additive isobolograms would be linear and designed a general equation (eqn. 1) which produces linear isobolograms without regard to the shape of the individual dose-response curves. Sühnel cites proofs contained in the 1985 Berenbaum publication and claims that they demonstrate that the assumptions used by our method (Prichard and Shipman Jr., 1990) are not always valid. These proofs, however, merely show that our method typically does not produce linear isobolograms.

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We do not agree with either the line of thought proposed by Berenbaum or his general equation inasmuch as they are based on the premise that additive (zero interaction) isobolograms will always be linear. Isobolograms were developed initially as tools to describe 3-D surfaces (Loewe, 1953) and are equivalent to horizontal cross-sections through the 3-D dose-response surface (reviewed in Prichard and Shipman Jr., 1990). When a drug is used in mock-combination with itself, the resulting isobolograms are linear. However, the conclusion that all additive isobolograms are linear is unfounded. Even Loewe who first described isobolograms, doubted that this was the case (see Loewe, 1953). Isobolograms are linear in the special case of a drug versus itself because the isobologram is also a line of equal drug concentration. Using eqn. 1 as an example, the isobologram of a drug versus itself is linear not because eqn. 1 is generally applicable, but because in the special case of a drug versus itself:

$X = Y = x + y$.

In our opinion, the fact that Berenbaum's method yields linear isobolograms irrespective of the shape of the individual dose-response curves argues that his equations are inappropriate. The probability that two unrelated nonlinear functions will interact to produce a 3-D surface with linear horizontal cross-sections is remote. In general, nonlinear functions and relationships between nonlinear functions, as in the case of dose-response curves, are extremely complex and are generally unpredictable and unsolvable (Gleick, 1987; Stewart, 1989). Given this fact, a general equation relating drug concentration and effect to predict the combined inhibition of two drugs in combination probably does not exist.

Nevertheless, the concept of synergy has merit and can be applied to relevant problems. It is a concept which describes the interaction between two or more drugs to produce a desired effect. The analysis of this relationship is important in choosing optimal drug combinations. Although it is impossible to mathematically predict the interaction of two drugs, even with detailed information about the mechanisms of action, interpretations of the 3-D dose-response surface are meaningful. We have developed a system, partially outlined in our review, which describes the nature of the 3-D surface. Because the mathematical prediction of additive interactions is impossible, we assume that the two drugs will act according to target theory (dissimilar site assumption) and use this surface as an approximation of additivity. The additive dose-response surface (dissimilar site assumption) is calculated with the following equation:

$$Z = X + Y(1 - X) \tag{2}$$

with Z equal to the inhibition produced by the combination, and X and Y equal to the inhibition produced by drugs X and Y, respectively. This assumption relates the final effects of the drugs used individually and eliminates the need for a general mathematical relationship (which does not exist) between the combination of doses and the final effect. Our method quantitates and analyzes

statistically the interactions with respect to this assumption. There are inherent limits to target theory, but it is a reasonable approximation of additivity and the quantitation of the observed effects makes the comparison of drug combinations possible. It also could be argued that the binding of one inhibitor could preclude the binding of a second molecule and, consequently, the inhibition could be described by the relation:

$$Z = X + Y \tag{3}$$

again with Z equal to the inhibition produced by the combination, and X and Y equal to the inhibition produced by drugs X and Y, respectively, when used individually (similar site assumption). We feel that the dissimilar site assumption, however, is a generally more appropriate estimation of additive effects.

Our method subtracts the estimated additivity (using either eqn. 1 or 2) from the experimental dose-response surface to reveal regions of synergy and antagonism. The volume of the synergy peaks is a measure of the quantity of synergy observed and is in units of $\mu M^2\%$. This is, of course, the 3-D counterpart to the area under a dose-response curve ($\mu M\%$). Similarly, the volume of the inverted (i.e. negative) peaks is a measure of the antagonism.

We have shown that the equation proposed by Berenbaum (1985) is inappropriate inasmuch as it is based on the incorrect premise that all additive isobolograms are linear. The methods proposed by Sühnel and Greco et al. are based upon Berenbaum's equation and consequently should be used with caution. Additionally, these methods are not quantitative. The quantitation of synergy is essential. In order to identify useful chemotherapeutic combinations, it is imperative to characterize the relationship and ask: (1) how much synergy is produced? (2) at what concentrations and ratios of concentrations does this occur? and (3) at what concentrations (if any) are the drugs synergistically cytotoxic? Three-dimensional methods are essential to answer these questions. Our method is uniquely suited as it is the only one that quantitates statistically significant interactions.

References

Berenbaum, M.C. (1985) The expected effect of a combination of agents: the general solution. J. Theor. Biol. 114, 413–431.

Berenbaum, M.C. (1989) What is Synergy? Pharmacol. Rev. 41, 93–141.

Gleick, J. (1987) Chaos: making a new science. Viking Penguin Inc., New York, NY.

Greco, W.R., Park, H.S. and Rustum, Y.M. (1990) Application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-β-arabinofuranosylcytosine. Cancer Res. 50, 5318–5327.

Loewe, S. (1953) The problem of synergism and antagonism of combined drugs. Arzneim. Forsch. 3, 285–320.

Prichard, M.N. and Shipman, C., Jr. (1990) A three-dimensional model to analyze drug-drug interactions. Antiviral Res. 14, 181–206.

Stewart, I. (1989) Does God play dice?: the mathematics of chaos. Basil Blackwell Inc., Cambridge, MA, U.S.A.

Sühnel, J. (1990) Evaluation of synergism and antagonism for the combined action of antiviral agents. Antiviral Res. 13, 23–40.

Sühnel, J. (1991) Zero interaction response surfaces, interaction functions and difference response surfaces for combinations of biologically active agents (Submitted).