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Alternate drug interaction analysis

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The potency of a drug combination may be due merely to the sum of the potencies of the separate components, or the components may interact in some way to either potentiate or inhibit their combined potency when administered together. Classical pharmacological analysis tests the null hypothesis that the potencies of the components are additive. Rejection of the null hypothesis implies that the components interact in some way either to potentiate or to inhibit the potencies of the components. The classical analysis is explained through the use of an example.

The potency of a drug combination may be due merely to the sum of the potencies of the separate components, or the components may interact in some way to either potentiate or inhibit their combined potency when administered together. Classical pharmacological analysis attacks the problem by testing the null hypothesis that the potencies of the components are additive, that is, that there is no interaction. Acceptance of the null hypothesis, additivity, implies that the individual potencies of the components are unchanged (within sampling variation) when administered together in the combination. On the other hand, rejection of the null hypothesis implies that the components interact in some way either to potentiate or inhibit the potencies of the components.

Isaac and Isaac (1978) analyzed their data by multiple comparisons of the treatments of special interest. These same data will be used here to demonstrate that the classical interaction analyses of Finney (1971) and of Gaddum (1948) provide direct and powerful tests for interaction and incorporate statistical significance tests of the hypothesis of additivity.

THE STUDY OF ISAAC AND ISAAC

A placebo, two doses (.4 and .8 mg/kg) of d-amphetamine (dA), two doses (1.6 and 3.2 mg/kg) of methylphenidate (MP), and the combination (1.6 MP + .4 dA) were administered to a group of 12 rats in a double 6 by 6 Latin square arrangement of treatments. Their locomotor activity responses were measured in a special light-box arrangement that counted the broken light beams due to the movement of the animal. The results, as given in Table 1, were read from Figure 1 in Isaac and Isaac (1978).

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Table 1

Treatment	Dosage	Response$^{1/2}$
Placebo		8.8
d-Amphetamine (dA)	.4	14.5
	.8	17.8
Methylphenidate (MP)	1.6	12.7
	3.2	15.2
Combination	1.6 MP + .4 dA	15.2

Note–Dosages are given in milligrams of drug per kilogram of body weight.

THE DESIGN

Isaac and Isaac (1978) were interested in comparing the combination to the low doses of their respective components. But in this unique design, the high doses provide the clue to the classic analyses of Finney (1971) and Gaddum (1948). Notice that the low and high doses of MP are four times the low and high doses of dA, respectively. Thus in planning their experiment, Isaac and Isaac probably expected a relative potency of 4:1 because of previous work reported by Kallman and Isaac (1975). In the additive model, the basic meaning of this relative potency is that dA in the combination could be replaced with a fourfold dose of MP. Thus the combination (1.6 MP + .4 dA) could be expressed as an equivalent dose of MP, as follows, 1.6 + 4(.4) = 3.2 mg/kg, which was the high dose of MP. The combination expressed as an equivalent dose of dA would be (1/4) (1.6) + .4 = .8 mg/kg, which was the high dose of dA. Therefore, the response to the combination could be compared with the equivalent doses rather than with the actual doses of their separate components. In this unique design, the low doses are the actual components, whereas the high doses are the equivalent doses of the combination. Although the low doses permit some comparisons of special interest, it is the high doses that provide the information for the analysis for drug interaction.

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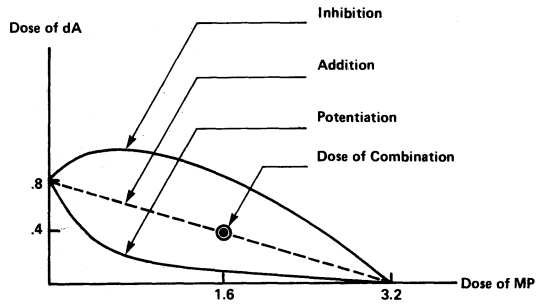

Figure 1. Gaddum plot test for interaction.

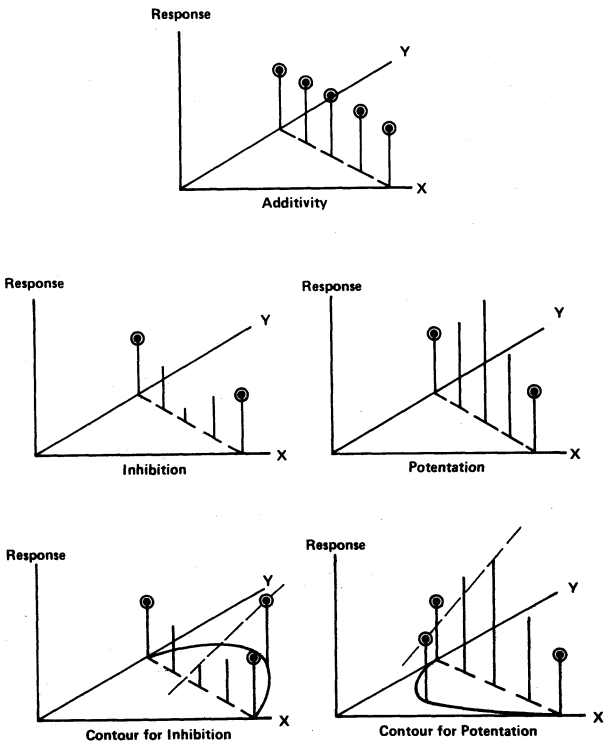

Figure 2. Contour curves for inhibition and potentiation.

In Gaddum's (1948) classical diagram (Figure 1), the equipotent doses of each component appear on the X and Y axes and are connected by a dotted line representing additivity. In Isaac and Isaac's (1978) remarkable design, the dose of the combination falls on this line.

The Gaddum (1948) plot needs some discussion. Visualize a monotonically increasing response surface above the X-Y dose plane. On this surface, draw any line of equal response levels. The projection of this line on the X-Y plane is a contour curve of equipotent doses. The contour curve for additivity is the straight line joining the equipotent doses of the individual components on the X and Y axes, whereas the contour curves for potentiation and inhibition must lie above and below this line, respectively. This is made clear in Figure 2, as follows. The dotted line is the line of equivalent doses for the hypothesis of additivity; equal potency is expected for all points on this line. If, however, the response to any combination on the dotted line is greater than the response at the endpoints of the line, then the contour line of equipotency must lie below the dotted line, since some lower dose of the combination would produce the equal response level. Such a contour line represents potentiation. Likewise, if the response to a combination on the dotted line is less than the response at the endpoints, then the line of equipotency must lie above the dotted line, since a greater dose would be required to obtain the equal response level, which represents inhibition.

All this appears to have been a part of the planning of the study. With this unique design, not only can the comparison of particular interest be made, but the broader question of interaction can be tested as well.

However, upon completion of the Isaac and Isaac (1978) study, the high doses did not produce equal responses. This situation has been resolved by Piserchia and Shah (Note 1), who have proved a theorem for the case of unequally potent endpoints that can be stated as follows: A response to a dosage point on the line of additivity greater than the maximum response obtained at the endpoints implies potentiation. In these data, the response to the combination was 15.2, whereas the maximum response was 17.8. Therefore, there was no potentiation of the individual components when administered in combination. In fact, inhibition was suggested.

Gaddum's (1948) graph can also be drawn for the observed data. Notice that the response to the combination was 15.2. Interpolating between the low and high doses of the separate components, one obtains the dose corresponding to the response level of 15.2, approximately .46 mg/kg for dA and 3.2 mg/kg for MP. Thus we can sketch Gaddum's graph (Figure 3). Notice that the contour line of equipotency lies above the dotted line, suggesting inhibition.

We have just discussed the classical method of Gaddum (1948) extended by Piserchia and Shah (Note 1) to cover the case of unequally potent endpoints. Piserchia

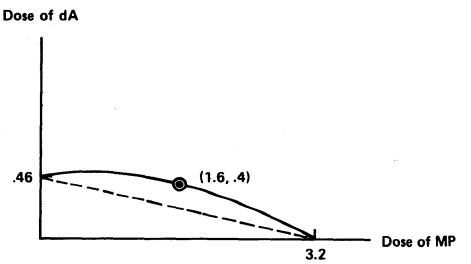

Figure 3. Gaddum plot of the data.

and Shah also provided a significance test for potentiation of toxicity data, utilizing the binomial distribution. We have written a similar significance test using the normal distribution, utilizing the error term from the analysis of variance. Since the error term was not given by Isaac and Isaac (1978), we cannot compute the statistics.

We will now turn our attention to the classical method of Finney (1971). Recall that a relative potency of 4:1 had been assumed in planning the experiment. However, after the study was completed, the potency of dA relative to MP was found to be 6.8 by fitting parallel lines to the data (Figure 4). Calculating the equivalent doses, using the observed potency of 6.8, one finds $1.6 + 6.8(.4) = 4.3$ of MP and $(1/6.8)(1.6) + .4 = .64$ of dA. The response corresponding to the dose of 4.3 of MP is 16.7. Likewise, the response corresponding to the dose of .64 of dA is 16.7. (See Figure 4.) This is the predicted response to the combination. The actual dose of the combination, 2.0 mg/kg, is obtained as the sum of the component doses, 1.6 of MP + .4 of dA. Hence, in Figure 4, the predicted response of 16.7 is plotted at the actual dose of the combination of 2.0. The dotted line through this point with the common slope of the parallel lines is the predicted dose-response line for the combination (Finney, 1971, p. 232, Equation 11.6). Recall the observed response to the combination was 15.2, which is also plotted in Figure 4. The dashed line plotted through this point with the common slope is the observed dose-response line for the combination. The observed line lies to the right of the predicted line, and this result indicates an interaction.

The relative potency, with 95% confidence limits, provides a significance test for the hypothesis of additivity. A relative potency of 1.0 represents additivity (i.e., the observed and predicted lines coincide). If the 95% confidence limits bracket 1.0, then the calculated relative potency does not differ significantly from 1.0 and the hypothesis of additivity (not interaction) is accepted. If, however, the 95% confidence limits do not bracket 1.0, then a statistically significant interaction exists; a relative potency greater than 1.0 demonstrates potentiation, whereas one less than 1.0 demonstrates inhibition. This is the classical method of Finney (1971). For Isaac and Isaacs' (1978) data, the relative potency, comparing the observed and predicted lines, is .71, which again suggests inhibition. But one needs the error term from the analysis of variance to calculate the 95% confidence limits.

Throughout this discussion, the placebo data were never mentioned. If a low dose of the combination with the same proportion of components (say, .8 MP + .2 dA) had been administered in place of the placebo, Isaac and Isaac (1978) would have had the ideal experimental

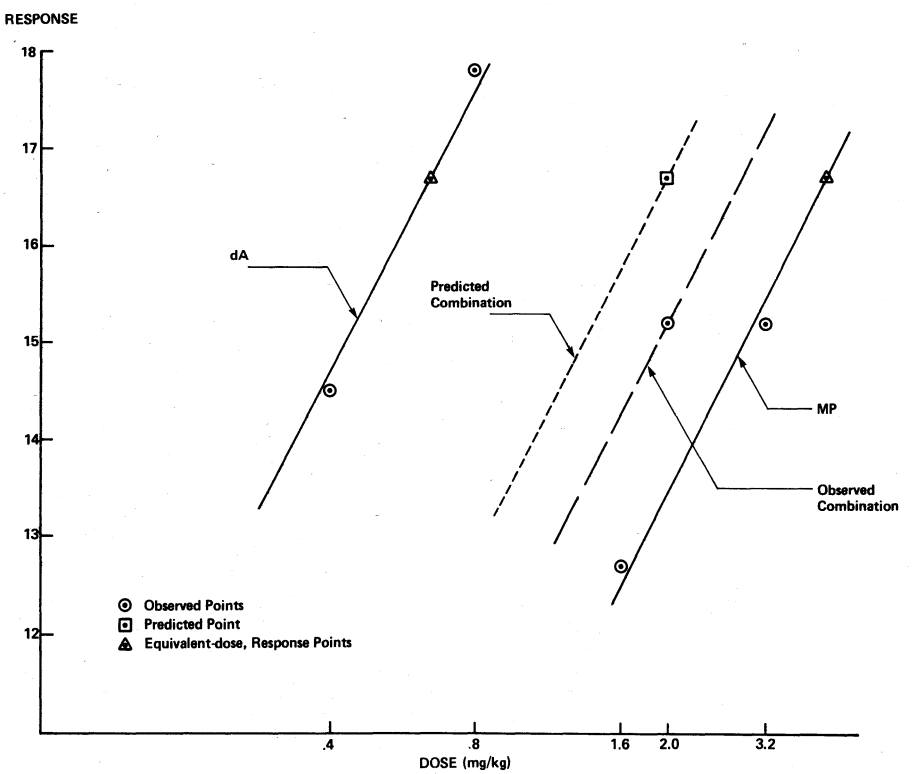

Figure 4. Parallel dose-response lines.

design. Parallel dose-response lines could then have been fitted to the combination as well as to the separate components and tested for deviation from parallelism. Assuming parallelism, the predicted line for the combination could have been calculated. Again, the potency of the observed relative to the predicted line for the combination could be calculated with 95% confidence limits. If the 95% confidence limits bracketed 1.0, then the hypothesis of additivity could be accepted. But if the 95% confidence limits did not bracket 1.0, then statistically significant potentiation or inhibition would be evidenced, according to whether the relative potency was greater or less than 1.0. This type of analysis would have provided far more information than the multiple-comparison analysis by utilizing the complete data set to obtain a single statistic (the relative potency comparing the observed and predicted lines for the combination) that could also be tested for statistical significance.

REFERENCE NOTE

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