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COMBINATIONS OF DRUGS THAT PRODUCE OPPOSITE EFFECTS

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Abstract: Numerous studies have examined combinations of drugs that produce overtly similar effects in order to determine whether such combinations produce unusual interactions indicating synergism. When two drugs, or a drug and an endogenous chemical, individually produce opposite effects there is equal interest in the quantitative assessment of interactions in these combinations. This case of opposing effects, not previously examined quantitatively, is the subject of this communication.

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1. Introduction

Quantitative information on drug action most often begins with tests that measure the drug's effect as a function of dose. An effect E is a measurable change in the system that accompanies drug administration in dose D. The dose-effect relation will, in general, have an upper limit $E_{\max}$ and is commonly described by the following hyperbolic relation

$$E = \frac{E_{\max} D}{D + C} \tag{1}$$

where C is a constant that is an indicator of the drugs potency. When drugs

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that individually produce overtly similar effects are used in combination there is interest in characterizing the combination, i.e., in determining whether an interaction between the two leads to an unexpected enhanced or reduced effect. Methods for examining drug combinations have a long history in pharmacological investigation and the methods employed have been amply described and summarized in several reviews [2], [7], [8], [9]. Some of these methods employ a graph called an isobologram that was introduced many years ago by Loewe [4], [5], [6] and this approach has been applied to numerous combinations involving two drugs (or two chemicals). Interestingly, combinations of drugs that produce opposite effects have received little or no attention. Here we are referring to drugs, other than competitive blocking drugs, that produce opposite effects (e.g., on heart rate, body temperature, pain perception, etc.) In this communication we address this question by presenting the quantitative methodology for determining interactions between two active drugs (called agonists) that individually produce dose-related opposite effects. The method is based on the concept of dose equivalence that is standard in analyzing combinations that yield similar effects. Hence we begin with a concise summary of the standard situation and then transition into the case of current interest.

2. Dose Equivalence

The standard method for analyzing drug combinations uses the concept of dose equivalence. In the simplest case each agonist drug is capable of achieving the same maximum effect and both drugs yield dose-effect data that are well fitted to an equation of the form (1). Denoting these drugs by A and B and their respective doses by a and b, we have $E = E_{\max}a/(a + C_A)$ and $E = E_{\max}b/(b + C_B)$. The ratio of equivalent doses is the constant $R = C_A/C_B$. Each dose a is therefore equivalent to a quantity of drug B given by a/R (and, clearly, a dose b of drug B is equivalent to bR of drug A). A dose combination (a, b) is assumed to be predictable by adding the dose of one and its equivalent from a dose of the other. Note that it is dose addition, and not effect addition, that is applicable to the combination. Using drug B as the reference drug we get the expected combination effect of (a, b)

$$E_{ab} = \frac{E_{\max}(b + a/R)}{(b + a/R) + C_B}. \tag{2}$$

Because this expected effect is due to addition of the dose of one agent and its equivalent from the other, we commonly refer to effects given by equation

(2) as "additive" and this outcome, if confirmed experimentally, suggests that there is no interaction between the two drugs. In testing actual combinations the effects calculated from (2) are compared with those that are experimentally obtained for the same dose combination and differences, if found, indicate either an enhanced or reduced effect due to some interaction. Enhanced activity, referred to as synergism, is especially interesting and important for both beneficial effects and toxic effects.

3. Isoboles

A common method of assessing synergism uses (a, b) curves of constant effect, thereby giving rise to level curves each of which corresponds to a specified effect magnitude. These curves, called isoboles, are convenient for display and also provide a basis for tests that distinguish additivity from non-additivity. Details of these tests are discussed in several previous communications [2], [7], [8], [9]. Most often the effect level used in the isobole is 1/2 of the maximum and thus, from (2), $b + a/R = C_B$, and since $R = C_A/C_B$, it follows that $a/C_A + b/C_B = 1$. More generally, for an effect level that requires doses A of drug A acting alone and B of drug B acting alone, the additive isobole is

$$\frac{a}{A} + \frac{b}{B} = 1. \tag{3}$$

The straight line given by the above equation is shown in Figure 1 along with experimentally derived dose combinations to illustrate additivity, sub-additivity and synergism. A dose combination that achieves the effect but does so with dose a pair that plos below the isobole is indicative of synergism; i.e., lesser quantities are needed to produce the effect. In contrast, a point above the isobole means that a sub-additive interaction has occurred. The linear isobole of additivity given by equation (3) is a consequence of the assumption that each drug is capable of achieving the same maximum effect. When the maxima differ the isobole is nonlinear [10] but its use in distinguishing synergism and sub-additivity from simple additivity is the same.

4. Drugs Exerting Opposite Effects

There are many situations in which an agonist drug is present along with a second agonist drug or an endogenous chemical that yields an effect that is opposite to that of the first. Lets say that it is drug A that produces the

positive effect and that it is present in a fixed dose a in the combination, while drug B exerts an effect in the opposite direction. Each drug is assumed to obey the hyperbolic dose-effect relation described by equation (1); thus, $E = E_B b/(b + C_B)$ for drug B and $E = E_A a/(a + C_A)$ for drug A, where E_A is positive and E_B is negative. The fixed dose a will have a dose equivalent of drug B, a dose that gives the same magnitude but opposite sign. We denote that nullifying dose by b_a. Following the same procedure used in isobolographic analysis the effect of the combination of this fixed a with variable b is therefore

$$E_{ab} = \frac{E_B(b - b_a)}{(b - b_a) + C_B}, \qquad b \geq b_a. \tag{4}$$

For quantities b that are less than b_a we refer these to their drug A-equivalent a_b; thus,

$$E_{ab} = \frac{E_A(a - a_b)}{(a - a_b) + C_A} \tag{5}$$

where

$$a_b = \frac{C_A}{\dfrac{E_A}{E_B}\left(1 + \dfrac{C_B}{b}\right) - 1}. \tag{6}$$

Equations (4), (5), (6) provide the expected (additive) effect for this situation. A computational example is provided below.

Example. For illustration we consider a combination of drug A yielding positive effects and drug B (with negative effects) according to the individual relations:

$$E = \frac{60a}{a + 50} \qquad \text{and} \qquad E = \frac{-100b}{b + 20}$$

where a is to be given in a fixed dose, $a = 50$. This quantity of a is equivalent to $b_a = 8.57$. Thus, for $b > 8.57$, the additive effect is given by

$$E_{ab} = \frac{-100(b - 8.57)}{(b - 8.57) + 20}.$$

For doses of $b \leq 8.57$ we calculate its drug A-equivalent, a_b, and get the additive effect from the drug A relation,

$$E_{ab} = \frac{60(a - a_b)}{(a - a_b) + 50}.$$

For $b = 0, 1, 2, 4, 6, 8$ the results are as follows:

b	a_b	E_{ab}
0	0	30.0
1	4.31	28.6
2	8.93	27.1
4	19.2	22.9
6	31.2	16.4
8	45.4	5.00

The above values of E_{ab} and those calculated for $b \geq 8.57$ are plotted in Figure 2.

5. Error Analysis

The rational function given by equation (2) contains the values C_A and C_B that are estimates from curve fitting and therefore have variances determined from the curve fit process. This is the common situation (two drugs with overtly similar effects) in drug combination analysis and, because of its widespread use, requires some discussion applicable to its use in comparing actual combination effects with those calculated from equation (2) that are simply additive. A more explicit form of that equation is

$$E_{ab} = \frac{E_{\max}(bC_A + aC_B)}{bC_A + aC_B + C_AC_B}. \tag{7}$$

Because the graph of equation (7) — or equation (2) — is a surface above the a-b plane, this form is sometimes referred to as a response surface. This form does not lead to an exact calculation of the variance of E_{ab}. Thus we apply the approximate method known as the "delta method" to estimate $Var(E_{ab})$ (see, for example, Cassella and Burger [3]). The formula for the delta method of approximating variances is derived from Taylor series and is expressed in conventional mathematics notation in terms of partial derivatives:

$$\begin{aligned} Var(f(x,y)) &= \left(\frac{\partial f}{\partial x}\right)^2 Var(x) \\ &+ \left(\frac{\partial f}{\partial y}\right)^2 Var(y) + 2\left(\frac{\partial f}{\partial x}\frac{\partial f}{\partial y}\right) Cov(x,y). \end{aligned} \tag{8}$$

We apply (8) to (7) and, because C_A and C_B are independent,

$$\begin{aligned} Var(E_{ab}) &= (bE_{\max})^2 \left(\frac{C_A}{bC_A + aC_B + C_AC_B}\right)^4 Var(C_B) \\ &+ (aE_{\max})^2 \left(\frac{C_B}{bC_A + aC_B + C_AC_B}\right)^4 Var(C_A) \end{aligned} \tag{9}$$

Statistical comparison between additive and experimental E_{ab} values are usually made with a set of (a, b) dose pairs yielding effects in the intermediate effect level. Statistically significant differences between additive and experimental values indicate departures from additivity.

The principal aim of this communication is the case applicable to drugs with opposite effects and the additive effect for this case is expressed in equations (4), (5), (6) which also contain random variables (such as b_a ,C_B, a_b and C_A). Again these forms do not lead to exact variance calculations for E_{ab} and, thus, we apply the delta method to these cases also. For example, when applied to (4) we get

$$\begin{aligned} Var(E_{ab}) &= \left(\frac{E_BC_B}{(b - b_a + C_B)^2}\right)^2 Var(b_a) \\ &+ \left(\frac{E_B(b_a - b)}{(b - b_a + C_B)^2}\right)^2 Var(C_B) \end{aligned} \tag{10}$$

Equation (10) allows an estimate of the variance of Eab for a combination containing a constant value a and varying b and is used for statistical comparison of E_{ab} with the experimentally determined effect of the combination for values of b that are greater than its drug A-equivalent. A similar procedure is used to get the variance for lesser values of b as determined from (5) and (6).

6. An Application

There are many drug or chemical combinations that involve constituents that individually produce effects in opposite directions, e.g., on heart rate, blood pressure, hormone release, body temperature, etc. One example pertains to studies in our laboratories on the body temperature changes that result from opioids and other drugs. A pilot study underway by our group is examining the body temperature changes in the rat for morphine in combination with a cannabinoid known as WIN 55,212-2. Studies of the individual compounds in rats showed that morphine produces an elevation in temperature [1] whereas

the cannabinoid lowers body temperature. Our interest is directed toward combinations of these drugs which are often used recreationally. This experiment is presented here because it provides a suitable application of the topic of this communication and is especially useful for demonstrating how the theory applied to preliminary data can guide the further planning of drug combination experiments such as the morphine-cannabinoid experiment that is underway in our laboratory.

The experimental procedure involved the use of male rats that received either a saline control or drug via the intraperitoneal route. Body temperature was monitored continuously (biotelemetry system) following drug administration (at time $t = 0$) and the drug effect was determined from the area under the temperature-time curve (AUC) for the time interval 30-60 minutes. Morphine (here denoted Drug A) produced an elevation of temperature whereas the cannabinoid (Drug B) produced decreases in temperature. The magnitude of these negative changes in temperature resulted in a dose-effect relation (AUC against dose) that was well fitted to a linear relation given by $E = 13.4b + 0.913$ for doses b over the range 0 — 6 mg/kg Higher doses that would ultimately display an upper limit were not used in this preliminary study. In contrast, morphine, tested in three doses, 0.1, 4.0 and 15 mg/kg produced positive effects (AUC) of 8.32, 32.2 and 35.0, respectively, thereby demonstrating and upper limit such as the typical hyperbolic relation previously described. It was decided to experiment with the two lower doses of morphine, each in a fixed amount in combination with increasing doses of WIN 55,212-2. Morphine dose 0.1 is equivalent to $b_a = 0.553 \pm 0.30$ of the cannabinoid, while morphine 4.0 is equivalent to $b_a = 2.33 \pm 0.23$. The standard errors of each b_a are noted here since these arise from the regression line as estimated mean values, and each tested b dose is reduced by the estimated ba value. Each actual b value, appropriately reduced by the estimated b_a is used in the WIN regression as $E = 13.4(bb_a) + 0.913$ to yield the estimated combination effect E_{ab}. While b is an administered dose and therefore assumed to be error free, the equivalent b_a has a variance that affected the estimated E_{ab}. Table 1 summarizes these results and also shows our very preliminary experimental results (E_{exp}) for the combinations tested thus far. The limited experimental data precludes a calculation of variances and, thus, no conclusion on possible interactions is made.

	WIN55,212-2 dose	E_{ab}	E_{exp}
Morphine 0.1	1.0	-6.95 ± 5.8	-12.9
	2.5	-27.0 ± 5.1	
	5.0	-60.5 ± 6.4	
Morphine 4.0	2.5	-3.19 ± 5.5	
	4.0	-23.3 ± 4.5	
	5.0	-36.7 ± 4.5	-25.3

Table 1: Additive and experimental effects of combinations of morphine and WIN55,212-2 on body temperature in the rat for i.p. doses (mg/kg).

7. Summary

Studies aimed at assessing interactions between drugs that are present together represent an important topic in pharmacology and therapeutics. Interestingly, little or no attention has been given to active drugs that individually produce effects in the opposite direction. That methodology, presented here, is modeled in a manner that is the same as that used for combinations of overtly similar drugs. The dose-effect modeling equations are rather simple (rational and linear functions) and apply well to many pharmacological agents. In cases in which alternate models apply, the fundamental concept of dose equivalence used here will still apply although these would present interesting new challenges in assessing variance estimates. Our hope is that this communication will stimulate interest in pharmacological modeling among mathematicians and others who read this journal.

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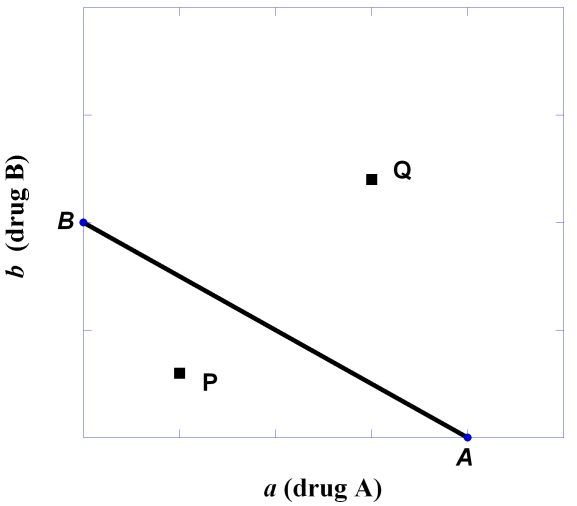

Figure 1: The isobole is shown as the straight line determined from the dose A of drug A and dose B of drug B that individually give an effect of specified magnitude. All points on the isobole are dose pairs that give the specified effect in the absence of interactions between the drugs, a situation of simple additivity that follows from dose equivalence (see text). Dose pairs off the line represent departures from additivity, e.g., point P below the isobole shows a dose pair that achieves the effect with lesser quantities. In contrast, point Q shows a situation requiring greater doses of the constituents. Point P indicates synergism (a positive and enhanced interaction) while point Q denotes a situation of sub-additivity.

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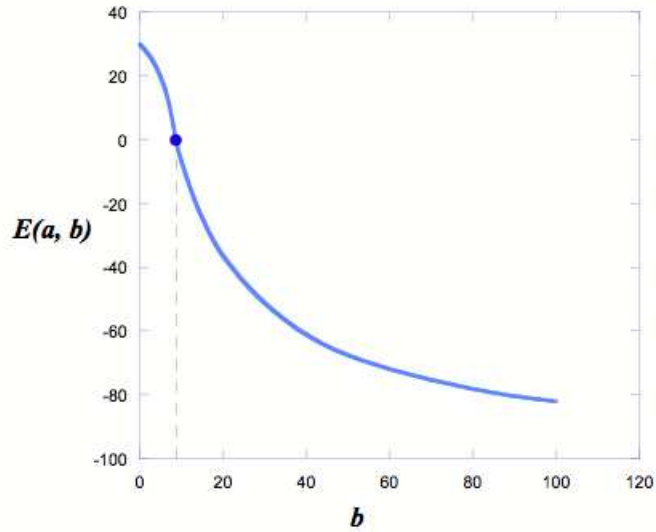

Figure 2: The effect of a fixed quantity of drug A that alone yields positive effects is present in combination with varying doses (b) of drug B that exerts negative effects. The point at $E(a, b) = 0$ and vertical line shown indicate the dose of drug B that nullifies the effect of the selected fixed dose of drug A.

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