Isobolographic Analysis for Combinations of a Full and Partial Agonist:

Curved Isoboles

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ABSTRACT

Combinations of drugs are frequently used therapeutically in order to achieve an enhanced effect without using an excess quantity of either agent. If the drugs exert overtly similar action, e.g. two analgesics, the effect of the combination may be tested for additivity, i.e. an effect level that is achieved based on the individual drug potencies. But combinations of agonists will sometimes display either superadditive (synergistic) or subadditive responses. Whether the two agonists are both drugs, or a combination of a drug and an endogenous chemical, there is interest in characterizing the interaction to determine whether it departs from additivity because quantitative information of this kind, aside from its therapeutic importance, may also illuminate mechanism. A common method for this characterization uses the isobologram. This is a plot in rectangular coordinates of dose combinations \((a,b)\) that produce the same effect level (often taken to be 50% of the maximum). In its usual form this plot is constructed as a straight line (of additivity) connecting intercepts that represent the individually effective doses, e.g., \(ED_{50}\) values of each. This line is the reference for distinguishing additive from non-additive interactions according as the tested combination is on or off this line. Discussed here are the assumptions that underlie this linear plot. Specifically we show that a combination of drugs with a variable potency ratio, exemplified by a full and a partial agonist, lead to curvilinear isoboles of additivity that may erroneously be attributed to either synergism or sub-additivity.
When two drugs produce overtly similar effects, e.g., two analgesics or two antihypertensives, their presence together may produce an effect whose magnitude is different from that predicted by the individual drug potencies. Combinations that achieve predictable effects based on individual potencies are said to be additive. Determining the additive effect for a drug combination is the first step in detecting synergism. That determination is straightforward when the drugs act through different receptors and have a constant relative potency $R$, i.e., when equally effective doses have the same ratio over the range of effects being studied. The constancy of $R$ is implicit in most studies of combinations that use isobolographic analysis. In such studies the dose-response curves of the individual agents allow a determination of each drug’s dose that gives a specified effect such as the half-maximal effect. That dose, the $D_{50}$, (or $ED_{50}$ if the dose-response data are quantal) for each drug is plotted as an intercept on a Cartesian coordinate system in which the axes represent the individual drug doses, and the two intercepts define a straight line called the line of additivity. All points $(a,b)$ on this line are dose pairs, called additive isoboles, that give the half-maximal effect if the joint action is in accord with their individual potencies. Although an effect level at $50\% E_{\text{max}}$ is the most common, any other effect level up to the maximum can be used; thus, isoboles actually fill an entire region of the $(a,b)$ coordinate plane. The set of additive isoboles in the $(a,b)$ coordinate plane determine the additive effect of any combination and thereby allow a view of the effect in a three dimensional plot in the form of a surface whose height above $(a,b)$ denotes the effect. (See Tallarida, 2001). This means that each additive isobole defines the planar curve of constant elevation (effect magnitude) in this response surface plot.
When the isobole for a specified effect has been determined, the dose pair of the combination that experimentally produces this effect may plot as a point that is below or above the additive isobole line and thus indicate super-additivity or sub-additivity, respectively. The graph containing the additive line and the plotted experimental point constitutes a graph known as an isobologram.

Since its employment by Loewe (1953) the isobologram has been used in many studies of drug combinations (e.g., Cichewicz et al, 2003; Fairbanks and Wilcox1999; Hurley et al, 1999; Wei and Roerig,1998; Gerthoffer, W.T.,1996; Field et al, 2002; Bolan et al, 2002.) as well as in experimental designs that administer a single drug at two different sites (Yeung and Rudy, 1980). The popularity of the isobologram is undoubtedly related to the simplicity of constructing the line of additivity (linear under the assumption of constant relative potency) and the consequent visual assessment it affords when the experimentally-derived dose combination for the specified effect is plotted. That experimental dose combination, usually obtained from a regression analysis of the combination, is then compared statistically with the corresponding combination dose on the additive line to determine a significant difference. Those statistical tests and additional discussions of isobolograms may be found in the monograph by Tallarida (2000) and in previous reviews (Tallarida, 1992; 2001).

The main subject of this communication is the analysis of a drug combination displaying variable relative potency as is the case when two drugs (or ligands) yield different maximum effects, e.g., in experiments that employ a full agonist and a partial agonist. In this case the relative potency is not constant and we show here that the *isoboles are not straight lines*, a fact that has not been adequately addressed in the literature. Specifically,
we show here how the fitted dose-effect curves of the individual agents are used in the
calculation of the additive isoboles and, thus, the expected effect for any dose
combination. We begin by examining the usual situation of constant $R$ and subsequently
derive and apply the equations to data with variable $R$.

**Theory**

The two compounds, A and B, will have a constant potency ratio when the individual
dose-response curves are simple hyperbolas with the same maximum effect:

$$E = E_{\max} A / (A + A_{50}) \quad \text{and} \quad E = E_{\max} B / (B + B_{50}) .$$

In this situation, $R = A / B = A_{50} / B_{50}$. Thus, when the two compounds are present together
in quantities $a$ and $b$, it is possible to express the quantity $a$ as an equivalent of compound
B, denoted $b'$, and given by $b' = a/R$. For any specified effect that requires dose $B$
(alone) the combination equivalent is $b + b' = B$, or $b + a/R = B$. It is common to express
the latter in intercept form

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

a straight line with intercepts $A$ and $B$ for the specified effect (the additive isobole). We
now consider the case in which the maximum effects are different. For convenience we
will take the maximum of the higher efficacy agonist (B) to be 100 and denote the
maximum effect of the lower efficacy drug by $E_c$; thus

$$E = \frac{100B}{B + B_{50}} \quad \text{and} \quad E = \frac{E_c A}{A + A_c}$$
These are hyperbolas that appear S-shaped when the dose scale is calibrated logarithmically as in figure 1. The combination dose \((a, b)\) of drugs A and B is equivalent to the dose \(b + b'\) of drug B, where \(b'\) is obtained by equating
\[
\frac{100b'}{b' + B_{50}} = \frac{E_c a}{a + A_c}.
\]
Thus,
\[
b' = \frac{B_{50}}{\frac{100}{E_c} \left(1 + \frac{A_c}{a}\right) - 1}.
\]
The isobole for the specified effect (that which results from dose B alone) is given by
\[
b + b' = B,
\]
or
\[
b + \frac{B_{50}}{\frac{100}{E_c} \left(1 + \frac{A_c}{a}\right) - 1} = B.
\]
Every effect level corresponds to some value of \(B\) so that equation (2) defines a family of curves, the additive isoboles, in the \((a, b)\) coordinate plane. From the form of equation (2) it is clear that these are not straight lines. (See Fig. 2 and the illustration below). For levels of effect below \(E_c\) (the maximum of the lower efficacy drug) the curves are hyperbolic arcs that join the intercepts \((0, B)\) and \((A, 0)\), where \(A\) is the dose of drug A achieving the effect \(E < E_c\). In contrast, if the effect is greater than \(E_c\), then the isoboles become unbounded arcs of hyperbolas starting at \((0, B)\) and decreasing toward a horizontal asymptote. Further mathematical details are given in the appendix.
Results

Illustration: Full and Partial Agonists

We first provide an illustration using sample hyperbolic dose-effect curves having different maxima. We shall subsequently use actual dose-effect curves that are described by more complicated fitting equations. The first illustration, shown in figure 1, uses hyperbolas that appear sigmoidal because of the logarithmically calibrated dose scale. With a linear dose scale these are hyperbolas of the kind used above, and having the parameter values given in the figure legend. In this case the lower efficacy agent produces a maximum $E_c$ that is 60% of the full agonist’s value. In this first illustration we have used equation (2) to calculate the isoboles for an effect greater than $E_c$, viz., 70% of the system maximum, and for two lesser effects, 35% and 50%. The isobole for the 35% effect is anchored by the individually effective doses, $A = 112$ and $B = 8.08$, and is moderately curved (Fig. 2). The isobole for the 50% effect has intercepts $A = 400$, $B = 15$ and is visibly curved and concave upward. For the effect level 70%, which is above drug A’s maximum, the additive isobole is an unbounded hyperbolic arc that decreases toward a horizontal intercept as shown in Fig. 2.

Results of a Hypothermic Combination

If one of the two compounds lacks efficacy then the concept of additivity means that its concomitant use with the active compound results in no change in the dose-effect relation of the active compound. However, higher doses of the “inactive” compound may produce measurable effects and, thus, the use of the higher dose range presents a case of
variable relative potency. In that regard we illustrate with the use of data obtained in a previous study (Rawls et al, 2002). As will be shown, the data from that study also requires a more general curve-fitting procedure which, in turn, necessitated a generalization of equation (2). It is, therefore, a very useful illustration. That study examined the effect of the NMDA antagonist dextromethorphan (DXM) on the hypothermic response to WIN 55212-2, a selective cannabinoid agonist, in rats. The cannabinoid produced dose-dependent hypothermia in the dose range 1-10 mg/kg, i.m., whereas DXM evoked dose-dependent hypothermia only for doses equal to or greater than 30 mg/kg (i.m). [However, the lower doses of DXM (10 mg/kg) potentiated the hypothermic response to WIN 55212-2 (1, 2.5, or 5 mg/kg)]. We use that data here to illustrate the calculations for the additive effect of a combination of WIN 55212-2, the full agonist, and DXM in its higher dose range, where it produces modest dose-dependent effects.

The dose-effect data for the partial agonist, DXM, and the full agonist, WIN 55212-2, were not well described by simple hyperbolas and, thus, required equations of the form

\[ E = \frac{E_c A^q}{A^q + A_c^q} \quad \text{and} \quad E = \frac{E_B B^p}{B^p + B_{50}^p} \]

respectively, where \( p \) and \( q \) are curve-fitting parameters ("Hill coefficients"). These graphs are shown in figure 3. The term \( E_B \) is the maximum effect (previously expressed as 100%), while all other terms have the same meaning as previously defined in equations (2). In this case the isobolar equation corresponding to equation (2) is
Further mathematical detail is given in the appendix.

Parameters of these fits for the hypothermic data were as follows: $E_c = 1.58; q = 1.92; A_c = 65.8; E_B = 4.17; p = 1.73; B_{50} = 3.99$. Isoboles for several different effect levels, shown in figure 4, were calculated from equation (3). Because the theoretical maximum effect of DXM is 1.58, the additive isoboles for the two largest effects shown do not have intercepts on the horizontal axis. While this example was chosen mainly to illustrate the calculations, the additive isoboles derived provide a basis for future combination experiments with WIN 55212-2 that employ DXM in this higher dose range.

**Variable Relative Potency for Two Full Agonists**

The combination of a full and partial agonist presented above represents a clear example of a varying potency ratio, one that becomes extreme as the effect level approaches $E_c$. But a variable potency ratio also occurs with two full agonists. In order to illustrate the isobole equations (and their graphs) when two full agonists have a varying potency ratio, we have used the parameters from the previous hypothermic example but adjusted the efficacies to be the same. This was done to illustrate a common situation in which two full agonists have non-parallel regressions of effect on log dose. Toward that end we used values of drug B (the more potent drug) that give several different effect levels. Figure 5 shows the isoboles for these effects. It is seen that these are slightly curved and are typical of this situation of non-parallel regressions. Further mathematical detail for
this case is provided in the appendix. In contrast to the combination of drugs of different efficacies, this combination leads to isoboles that reach both axes and, because \( q \) and \( p \) differ only slightly, the curvature is minimal. When \( q/p > 1 \), as in this illustration, the isoboles (plotted as \( b \) against \( a \)) are concave downward. For \( q/p < 1 \), the isoboles are concave upward.
Discussion

Combinations of drugs or endogenous ligands in which the individual agents have a constant relative potency yield linear and parallel isoboles of additivity. This applies to two agents whose log dose-effect relations are parallel or to drug pairs whose dose-effect equations are simple hyperbolas with the same maximum. These conditions are implicit in typical studies of combinations and the isoboles so derived have been used to distinguish departures from additivity. However, when the constancy of $R$ cannot be assumed, as in the case of a full and partial agonist, the additive isoboles are not straight lines but are curves as demonstrated here. The isobologram is still useful in this case but its construction is a bit more complicated (see equations (2) or (3)). In these cases, if linear isoboles are (incorrectly) drawn and the actual data displays the theoretically correct concave-upward curve of additivity, it may be wrongly concluded that the data show synergism. Thus, special attention is needed when the two agents have obviously different efficacy values. When both compounds are full agonists with a variable potency ratio the additive isoboles are still anchored by intercept values $A$ and $B$ and have a curvature that depends on coefficients $p$ and $q$.

The term denoted by $\gamma$, defined in the appendix, has a relation to the relative potency that becomes more evident when the dose component $a$ of drug A is small. It is seen (for the simple hyperbolic case and low doses) that $\gamma$ is a non-dimensional parameter that indicates the relative potency of drugs A and B: one unit of drug B is equivalent to $\gamma$ units of drug A. More generally, one unit of drug B is equivalent to an amount of drug A given by $\gamma^{1/q}$. Most published reports that use isobolographic analysis have involved drugs that are equal, or approximately equal, in efficacy. In certain other studies one of
the drugs has zero efficacy, a case that is readily analyzed by comparing the dose-effect curve of the active agent alone and when it is in combination as previously described. A significant difference then indicates a departure from simple additivity. That situation applied to low doses of DXM given concomitantly with WIN 55212-2. Another early example is contained in a study by Porreca et al (1990) in which it was shown that peripheral [Leu5]Enkephalin had virtually no antinociceptive efficacy but, when administered with morphine, the combination enhanced morphine’s effects.

The combination of a full and a partial agonist, the main topic of this investigation, has not received adequate attention. The analysis presented here shows that this situation leads to curvilinear isoboles. That curvature could be misinterpreted to suggest synergism (or sub-additivity) when the combination is actually additive.

Additivity is based on the concept of dose equivalence, i.e., how much of one of the drugs is equivalent to a dose of the other. This equivalence is the basis of the relation derived for the additive isobole so that the combination doses, \( a \) and \( b \), can be expressed as a dose of either one of them. In our derivation we chose to demonstrate the conversion of dose \( a \) of drug A into an equivalent of drug B. Thus, dose \( b \) plus this equivalent becomes the dose of drug B that yields the expected effect when this summed dose is used in the dose-effect equation of drug B. All tested dose pairs provide additive effect values, thereby producing the additive dose-effect relation. This relation is then viewed against the actual dose-effect relation that is experimentally determined. The additive and actual dose-effect relations, expressed in the usual way as regressions of effect on log (dose), thereby provide the additive and the experimental doses for the effect level selected (usually 50% of \( E_{\text{max}} \)). These are statistically compared as described by Tallarida.
A significant difference indicates a departure from simple additivity, either synergism or sub-additivity depending on the relative values of the additive and actual dose values. Combinations of the kind discussed here, i.e., those with variable relative potency, are likely to occur in detailed studies of mechanism, especially in mechanistic studies involving endogenous compounds. For example, endogenous opioids (enkephalins, endorphins, and dynorphin) have been the subject of many studies of their ability to activate G proteins as measured by the binding of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ to membranes. Some of these compounds show high efficacy when compared to common agonists such as morphine and meperidine (Alt et al., 1998). Marked differences in both efficacy and potency have also been seen among mu opioids using forskolin-stimulated cAMP production as shown by Gharagozlou et al. (2000). That study demonstrated the rank of order of efficacy of certain compounds as follows: beta endorphin $>$ fentanyl $>$ etorphine $>$ morphine $>$ cyclazocine. In contrast, their potencies were different in rank order: etorphine $>$ cyclazocine $>$ fentanyl $>$ morphine $>$ beta endorphin. As more studies of intimate mechanisms are conducted it is likely that additional combinations involving endogenous and exogenous agents will be examined for interactions and, because of these marked differences in efficacy and potency, the analysis presented here is especially applicable. A further application is to studies involving a drug and its active metabolite(s). An example is afforded by buprenorphine which produces an active metabolite, norbuprenorphine, of lower efficacy (Ohtani et al., 1995; Cowan, 2003). The difference in efficacies requires the methodology presented here in order to detect possible interactions between the compounds, an area currently being pursued in our laboratories. Aside from these mechanistic-type studies there will continue to be interest
in joint drug application and, when these show varying relative potency, it is necessary to calculate the additive isoboles as described here (equations 2, 3). Because the additive isoboles define the expected response surface of a combination experiment this approach is quite useful in studying combination drug action. The use (misuse) of linear isoboles in cases of variable relative potency could lead to incorrect conclusions on the nature of the possible interaction of the agents.
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Footnote

1 Isoboles are analogous to contour curves used in describing hilly terrain.
Legends for Figures

Figure 1. Full agonist (B) with half-maximal-dose $B_{50}$, and a partial agonist (A) that attains a maximum effect $E_c$ (in this illustration = 60%) and half that maximum with dose $A_c$. $B_c$ is the dose of drug B that produces the maximum effect $E_c$ of drug A. Both dose-effect curves are standard hyperbolic curves that appear sigmoidal in the plot because of a logarithmically calibrated dose scale. The curves illustrated have the following values: $B_{50} = 15$, $A_c = 80$, and $E_c = 60$ and, from these, $B_c = 22.5$.

Figure 2. Isoboles for three effect levels for a combination of a full and a partial agonist illustrated in figure 1. For effect levels less than the maximum of the lower efficacy drug (A), in this case 60%, the isoboles are arcs of hyperbolas that have intercept values equal to the doses of the individual drugs that produce the effect. For an effect greater than 60%, the isobole is an unbounded arc that approaches the horizontal asymptote whose value is $B - B_c$ (in this case, 12.5 for the 70% isobole). See the appendix for details of the calculation.

Figure 3. Dose effect curves for the decrease in body temperature produced by DXM and WIN 55212-2. These were derived using curve-fitting parameters ($q$ and $p$) as described in the text. (Data from Rawls, et al, 2002).

Figure 4. Isoboles for selected effect levels (temperature decrease) for the drug combination shown in figure 3. For doses of WIN 55212-2 greater than 3 mg/kg the isobole of additivity is an unbounded arc that approaches a horizontal asymptote.
Figure 5. Illustration using the $p$ and $q$ values indicative of a variable potency ratio for two full agonists. Toward that end we took the $p$ and $q$ values for the DXM and WIN hypothermic data ($p = 1.73; q = 1.92$) but, to illustrate the concept, adjusted each to the same maximum effect, 4.17, so that $A_c = A_{50} = 65.8$ and $B_{50} = 3.99$. This choice produced the slightly curved isoboles for the effect levels (labeled A to G) shown.
Isoboles for effect levels 35%, 50% and 70%
Effect levels, Temp drop, °C

A: 0.5,  B: 1.0,  C: 1.5 ...  G: 3.5
APPENDIX

Hyperbolic dose effect curves with different maxima

Let $B_c$ be the dose of drug B that produces the maximum effect $E_c$ of drug A. Then the additive isoboles given by equation (2) may be written

\[
B + \frac{1}{\frac{1}{B_c} + \frac{\gamma}{a}} = B
\]  

(A-1)

where

\[
\gamma = \frac{100A_c}{E_c B_{50}}
\]

For levels of effect greater than $E_c$, $B \geq B_c$, and the additive isobole starting at $(0,B)$ is an unbounded hyperbolic arc that decreases toward the horizontal asymptote $b = B - B_c$.

When $B \leq B_c$, meaning effect levels below $E_c$, the additive isoboles are arcs that join intercepts $(0,B)$ and $(A,0)$ where $A$ and $B$ are unitary doses that are equally effective.

Intercept $A$ may also be expressed by

\[
A = \gamma \left( \frac{1}{B} - \frac{1}{B_c} \right)^{-1}
\]  

When written this way an interpretation of $\gamma$ is evident; it is seen that for very small doses, hence very small values of $A$ and $B$, this expression gives

$A = \gamma B$, thereby illustrating that $\gamma$ is a relative potency term for small doses; i.e., one unit of drug B is equivalent to $\gamma$ units of drug A.

Curve-fitting parameters
For the hypothermic combination, which uses curve-fitting parameters \( p \) and \( q \), the form given by Eq. (A-1) becomes

\[
\frac{1}{b + \left( \frac{1}{\left( \frac{1}{B_c^p} + \frac{\gamma}{a^q} \right)^p} \right)^{1/p}} = B
\]

(A-2)

where \( \gamma = \frac{E_B A_c^q}{E_c B_{50}^p} \). In this case we also get an interpretation of \( \gamma \) by considering very small doses: \( A^q = \gamma B^p \). For effects \( \geq E_c \) the additive isobole starts at \((0, B)\) and is an unbounded arc that decreases toward the asymptote given by \( b = B - B_c \). For effects \( \leq E_c \), the additive isobole originates at \((0, B)\) and terminates at \((A, 0)\) where intercept \( A \) is given by

\[
A = \left[ \frac{\gamma}{\left( \frac{1}{B^p} - \frac{1}{B_c^p} \right)} \right]^{1/q}
\]

**Two full agonists with different regression line slopes**

A compound whose dose-effect data are modeled with a curve fitting parameter \( p \)
according to \( E = \frac{100B^p}{B^p + B_{50}^p} \), commonly has the data displayed as \((\log B, E)\) and these data are fitted by a linear regression of \( E \) on \( \log B \). (Common logarithms are most often used.) This transformation of dose to the logarithmic scale leads to the “logistic” form:
\[ E = \frac{100.10^x}{10^x + B_{50}^p} \quad (A-3) \]

where \( x = \log(B) \).

This common procedure, when limited to effects in the midrange, has a slope that is proportional to \( p \). Specifically, at the 50 % effect level it may be shown that the slope = 25 \( \ln(10) \) \( p \) (approximately 57.6 \( p \)). Thus, two full agonists with different curve-fitting parameters, \( p \) and \( q \), will have regression line slopes that differ. This situation is illustrated in the example described in the text and illustrated in figure 5. In this case each maximum is normalized to 100 and thus \( A_c = A_{50} \). The isobole equation corresponding to equation (A-2) becomes

\[ b + \frac{a^{q/p}}{\gamma^{a/p}} = B \]

where

\[ \gamma = \frac{A_{50}^q}{B_{50}^p} . \]

Thus

\[ b + B_{50} \left( \frac{a}{A_{50}} \right)^{q/p} = B \quad (A-4) \]

Equation (A-4) defines the family of isoboles for this case of two full agonists. When plotted as \( b \) against \( a \) and analyzed with the second derivative the isoboles are seen to be concave upward for \( q/p < 1 \) and concave downward for \( q/p > 1 \).