
Perspectives in Pharmacology

Revisiting the Isobole and Related Quantitative Methods for Assessing Drug Synergism

Ronald J. Tallarida

Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, Pennsylvania

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ABSTRACT

The isobole is well established and commonly used in the quantitative study of agonist drug combinations. This article reviews the isobole, its derivation from the concept of dose equivalence, and its usefulness in providing the predicted effect of an agonist drug combination, a topic not discussed in pharmacology textbooks. This review addresses that topic and also shows that an alternate method, called “Bliss independence,” is inconsistent with the isobolar approach and also has a less clear conceptual basis. In its simplest application the isobole is the familiar linear plot in Cartesian coordinates with intercepts representing the individual drug potencies. It is also shown that

the isobole can be nonlinear, a fact recognized by its founder (Loewe) but neglected or rejected by virtually all other users. Whether its shape is linear or nonlinear the isobole is equally useful in detecting synergism and antagonism for drug combinations, and its theoretical basis leads to calculations of the expected effect of a drug combination. Numerous applications of isoboles in preclinical testing have shown that synergism or antagonism is not only a property of the two agonist drugs; the dose ratio is also important, a fact of potential importance to the design and testing of drug combinations in clinical trials.

Introduction

In this article I review the isobologram, a graphical method introduced many years ago for the purpose of assessing synergism or subadditivity for agonist drug combinations. The isobole approach is applicable to pairs of drugs that produce overtly similar and measurable effects that have been tested as a function of their respective doses or concentrations. This method has been used to analyze experiments in whole animals and isolated tissue preparations and in studies of more intimate action at the cellular level.

For more than two drugs the familiar isobolographic approach is not applicable. However, the theory that underlies the isobole is still applicable, even though the graph may not be. In that regard, I use the concept of dose equivalence, from which the isobole is derived, and examine combination effect levels, a topic discussed in a later section (*Calculating the Combination Effect*).

This article follows my earlier *Perspectives*, but in writing this one I have an additional purpose, namely to show that this approach is useful for developing optimal combinations of fixed-dose combinations. In addition, I will show how the theoretical basis of the isobologram relates to another method for studying drug combinations, one that views the results on the effect scale, i.e., what is the expected effect of a dose combination? The isobole approach and the effect-scale approach are completely consistent in determining synergistic and antagonistic interactions and stand in contrast to another effect-scale method (Bliss independence) that gives different results. I have kept the mathematical aspects to a minimum. Instead, I use selected examples to illustrate the main points. The isobole approach is popular because it is easy to use and very easy to use in many circumstances, i.e., in those cases where the pair of drugs has a constant, well defined potency ratio. In addition, the theoretical basis of its derivation and usage is quite clear as I will show here.

I begin by asking, “What is an isobole?” As introduced by Loewe (1927, 1928, 1953), it is a graph in Cartesian coordinates in which the axes are the doses of the respective drugs. These are drugs or compounds that produce overtly similar effects (e.g., two analgesics, two antihypertensives, etc.) The graph consists of a line (or a curve) that represents dose pairs,

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used together, that give a specified effect level if the drugs act independently. Each axial intercept denotes the dose of the drug when it is the sole agent. Therefore, if the effect selected is half the maximum effect (E_{max}), then the intercepts are the

ED_{50} values. It is convenient to denote the drugs as drug A and drug B. Figure 1 provides an illustration. This illustration is for the case in which drug B and drug A individually attain the maximum effect, have a constant potency ratio, and have

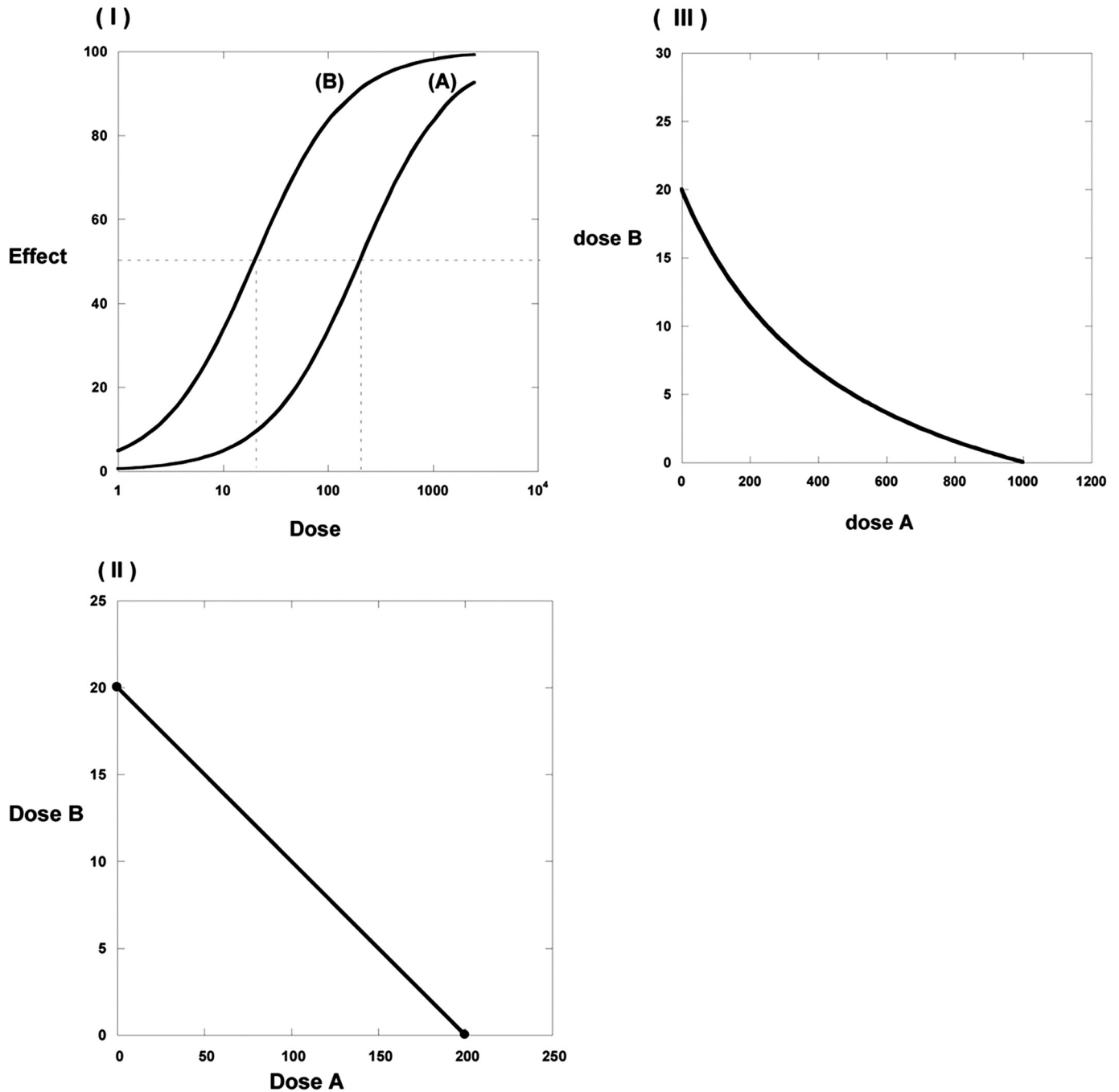


Fig. 1. I, dose-effect curves for agonists with a constant potency ratio (10:1) with ED_{50} values of 200 and 20. II, the isobole for the 50% effect is linear, has intercepts equal to the respective ED_{50} values, and obeys the equation $a/200 + b/20 = 1$ for doses a and b . All points (a, b) on the isobole represent dose pairs that are expected to give effect = 50% E_{max} when there is no interaction between the drugs. III, if the maximal effects E_A and E_B differ (say, $E_A = 60$ and $E_B = 100$), the potency ratio is not constant and the isobole, for the usual hyperbolic dose-effect curves, is nonlinear and given by the equation (Grabovsky and Tallarida, 2004):

$$b = ED_{50}(B) - \frac{ED_{50}(B)}{\frac{E_B}{E_A} \left\{ 1 + \frac{C_A}{a} \right\} - 1}$$

where C_A is the dose of drug A that gives effect $\frac{1}{2} E_A$. For dose-effect curves with slopes that differ this result is easily generalized as discussed in Grabovsky and Tallarida (2004) and Tallarida (2006, 2007).

ED₅₀ values of 200 mg for drug A and 20 mg for drug B. Thus, the potency ratio at all effect levels is 10 to 1. Before describing the isobole mathematically, we note that this line has a negative slope. This is intuitively clear because the presence of a quantity of drug A reduces the need for drug B (and vice versa) in reaching the specified effect level. It is obvious that when drug A is present at 200 mg the quantity of drug B needed is zero, and when drug B is present at 20 mg the quantity of drug A needed is zero. The isobole so derived is based entirely on the relative potency and says nothing about mechanism, only that the drugs, in combination, act in accord with their individual potencies. The legend of Fig. 1 provides further detail. The isobole is the locus points (dose pairs) that are expected to give the specified effect (e.g., $\frac{1}{2} E_{\max}$) when there is no interaction between the constituents, and it is derived from the parent dose-effect curves. (This derivation is as simple as determining how much of an artificial sweetener can be substituted for a quantity of sugar in a recipe.) As I will subsequently show, the concept of dose equivalence that is the basis of the isobole leads to other approaches for assessing the expected effects of a drug combination.

Use of the Isobole

How is the isobole used? This question leads to a discussion of the experimental design for assessing interactions such as synergism and subadditivity. To begin, the individual dose-effect data for each drug are plotted and fitted to a smooth curve (usually by regression techniques). From this pair of curves are derived the doses of each (individually) that give the effect of interest, which is usually $\frac{1}{2} E_{\max}$. These are denoted here by A for drug A and B for drug B. When the potency ratio (ratio of doses that individually give the same effect) is constant at all effect levels the isobole is a straight line connecting the axial intercepts at A and B. (In a later section, *Deriving the Isobole*, I discuss the case of a varying potency ratio.) All points on the isobole represent dose pairs that are expected to give the specified effect when there is no interaction. Because the isobole is derived for a specific effect level, an experiment with actual dose combinations is aimed at determining the combination dose pair that gives that specified effect. For example, say that the effect is 50% E_{\max} . How does one experimentally determine the (a,b) combination that actually gives the 50% effect? This requires that a set of doses that preserves some constant dose ratio be administered. This procedure yields dose-response data that are subjected to curve fitting (such as regression), and from the smoothed dose-response curve the dose combination that gives the 50% effect is obtained. If there is no interaction this observed dose combination is theoretically a point on the isobole (or not significantly off the isobole). If that observed point is off and below the isobole, there is synergism; if it is off and above the isobole there is subadditivity (Fig. 2). Both the observed point and the corresponding point on the isobole are estimated mean values with S.E. Therefore a conclusion regarding a point being off or on the isobole requires statistical analysis, a topic that has been described previously (Tallarida, 2000, 2006, 2007; Tallarida and Raffa, 2010). It should be noted that synergism is not merely a property of the two drugs; it also depends on the dose ratio. In preclinical testing the dose ratio most often tested is the ratio of the ED₅₀ values of the two drugs. A

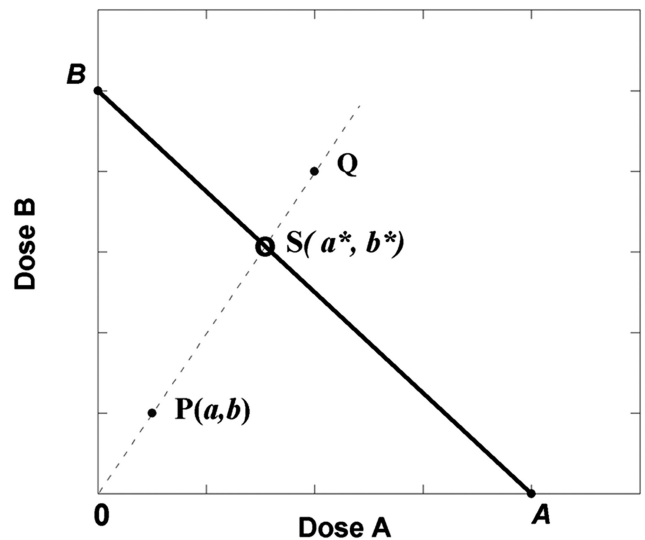


Fig. 2. Shown here is a linear isobole with an observed synergistic point P having coordinates (a,b), the additive point S with coordinates (a*,b*), and a subadditive point Q. The broken radial line denotes the dose combinations in a fixed ratio. In this case of a linear isobole the interaction index, denoted T , is calculated from the coordinates of P as $T = aA + bB$, a value less than unity and therefore indicative of synergism. For this case (linear isobole) it also follows that T is the ratio of radial distances OP/OS , which also equals the ratio of total doses, $(a + b)/(a^* + b^*)$. Situations of suspected departures from additivity require statistical testing of the difference (observed total – additive total). The variance (square of S.E.) for the additive total Z_{add} is given by

$$\text{Var}(Z_{add}) = \left[\frac{p_1 Z_{add}^2}{A^2} \right]^2 \text{Var}(A) + \left[\frac{p_2 Z_{add}^2}{B^2} \right]^2 \text{Var}(B).$$

The S.E. of the observed total dose is obtained from the dose-effect data of the combination. These totals and their S.E. are examined as the difference as described previously (Tallarida, 2000).

reasonable follow-up is to use 2:1 and 1:2 ratios of the ED₅₀ values and thereby get the observed dose combinations (points) that gave the 50% effect for these dose-ratio combinations. This choice of dose ratios is symmetric around the 1:1 ratio and may show additivity or a departure from additivity. Other ratios such as 3:1 and 1:3 might also be tested, thereby scanning the radial sector defined by the axial intercepts to detect other possible departures from additivity. The different dose ratios may not all be synergistic (or antagonistic), but among those that are synergistic one can assess the degree of synergism from the interaction index (Tallarida, 2002) which is described in the legend to Fig. 2. [Fig. 2 also shows a radial line that represents a particular dose ratio that is tested and found to have an observed dose pair, point P, that is below the additive dose pair (point S).]

The isobole approach is well established and quite commonly used. It seems to have gained its popularity when Gessner and Cabana (1970) used it to assess toxic (loss of righting reflex) and hypnotic interactions between chloral hydrate and ethanol in mice. Since then, many other investigators have applied this method. Adams et al. (1993) applied it in a study of opioid μ and δ agonists in the rat hot water and tail-flick tests of antinociception. Fairbanks and Wilcox (1999) examined antinociceptive combinations of morphine and clonidine in mice by using the hot water test. Hammond et al. (1998) studied antinociceptive combinations of δ -1 and δ -2 opioid receptors in rats by using spinal and supraspinal dosing after formalin-induced pain. Locomotion

was the endpoint in a study by Kimmel et al. (1997) that used combinations of buprenorphine and cocaine in rat. Antinociceptive combinations of morphine and clonidine have also been tested by using both the tail-flick and hot water tests in the rat (Ossipov et al., 1990) and the hot water test in the mouse (Tallarida et al., 1999). Wilcox et al. (1987) examined this same combination by using motor and sensory responses in the rat. [Leu5]-enkephalin, which has no detectable antinociception action in mice, was studied by Porreca et al. (1990) for its combinations with morphine in the hot water test. Raffa et al. (1993), in a most interesting study, tested combinations of the enantiomers of tramadol in an effort to further understand the coexistence of the opioid and non-opioid component of its analgesic action. After the determination of the affinities of the (+) and (−) enantiomers, Raffa et al. studied the antinociceptive action of each in the mouse abdominal constriction test and then compared the additive potency with that of racemic tramadol. Synergy was found, which was further confirmed in the mouse 55° hot water test and the Randall-Selitto yeast-induced inflammatory nociception model. Especially interesting is that these enantiomers interacted less than synergistically in certain side effects such as colonic transport inhibition.

Rawls et al. (2002) studied combinations of cannabinoids in producing hypothermia in rats that received the drugs directly into the hypothalamus. Woolverton et al. (2008a,b) tested various agonist combinations, including cocaine and remifentanyl, because these affect self-administration in monkeys. Codd et al. (2008) combined anticonvulsants and tramadol in a test of antinociception that was also analyzed with isobolograms. In these allodynia (in the rat) was produced surgically with nerve ligation. Tramadol, in combination with each of two anticonvulsants tested, showed antinociceptive synergism. Tanda et al. (2009) examined combinations of cocaine and other dopamine uptake inhibitors in mice. The effect of interest in these tests was the change in dopamine levels in the shell of the nucleus accumbens. Another interesting application of isoboles was made in a study of glucosamine and ibuprofen in combination in the mouse abdominal constriction test. Glucosamine, which lacks acute analgesic efficacy, markedly enhanced the potency of ibuprofen when the combination was used, a result indicative of pronounced synergism (Tallarida et al., 2003). In this case, where one drug lacks efficacy, the 50% isobole is horizontal and is positioned at the ED₅₀ level of the active drug.

Isobolographic analysis, and the concept of dose equivalence on which it is based, has been further applied in several more recent studies. Schröder et al. (2011) studied the two components of the analgesic action of tapentadol (μ -opioid interaction and inhibition of norepinephrine reuptake) by conducting tests in rodents by using both the tail-flick and nerve ligation models for measuring antinociception. That study used specific blockers of each component (naloxone to block μ opioid and yohimbine to block the inhibitory norepinephrine reuptake). Thus the μ -opioid effect versus dose and the adrenergic effect versus dose were obtained. This pair of dose-effect curves allowed an isobolographic analysis. A related study by Christoph et al. (2011) examined a combination of pregabalin and tapentadol in a rat model of neuropathic pain. Iwuchukwu et al. (2011) tested resveratrol in combination with other dietary polyphenols by using as the effects the enhanced antiproliferation and UDP-glucuronosyltransferase 1A1 induction in Caco-2 cells. Boehm et al.

(2010) applied isobole methods in a study of combinations of midazolam and dexmedetomidine in the rat tail-flick test of antinociception. My colleagues and I further extended the dose-equivalence methodology to design and analyze experiments with drugs that produce opposite effects and provided the mathematical detail (an application of dose equivalence) in a theoretical paper (Tallarida et al., 2011). Related to this is another study (Tallarida et al., 2010) that applied the methodology to the inverted U-shaped dose-effect curve of buprenorphine that was determined in both the rat and mouse hot water models of nociception. The conclusion of this study is that there is a second component of buprenorphine's action that probably is related to nociceptin/orphanin-FQ activation. This line of research applied in a study of vascular smooth muscle (aorta) of the rat (Lamarre et al., 2011) that examined tension development caused by angiotensin II and endothelin-1 measured individually. The dose-effect data were measured for isometric tension development in both endothelial-denuded and intact vessels. The dose-effect relation of the endothelial relaxing component of each agonist (most likely nitric oxide), which should not be calculated from simple effect subtraction, was derived by the method of dose equivalence, the same concept that underlies the isobole. The citations mentioned here represent only a very small fraction of the numerous studies that have used the isobolographic procedure.

Deriving the Isobole: Why Is It Termed “Additive?”

The expected (additive) effect of a combination depends on the individual dose-effect curves, which may, on a logarithmic scale, be parallel or nonparallel. To illustrate, I consider the case in which the drugs have a constant potency ratio (the parallel case) and individual ED₅₀ values, which, for convenience, are denoted here by *A* and *B*. A constant potency ratio means that at every level of effect the equally effective doses of drug *A* and drug *B* have the same dose ratio. I now consider a combination (*a*,*b*) that will give the 50% effect. To derive the isobole I first convert dose *a* to its drug *B* equivalent, a calculation easily made from the dose-effect curves of the individual drugs. Because the potency ratio is constant this drug *B* equivalent is (*a B/A*). The quantity of drug *B*, denoted *b*, and the drug *B* equivalent of dose *a*, denoted beq(*a*), must be added to give *B*. Thus, *b* + beq(*a*) = *B*, and because beq(*a*) = *a B/A*, we have *b* + *a B/A* = *B*, which simplifies to

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

Equation 1 is the familiar linear isobole. It is a consequence of the constant potency ratio *A/B*. It is termed “additive” because of the addition of dose *b* and the drug *B* equivalent of dose *a*. The term additive does not mean “effect of *a* + effect of *b*.” The conceptual basis of the isobole is quite clear and leads to the above straightforward derivation, which is easier to follow than other models with less clear bases. The additive isobole so derived gives the expected combination dose, and this is then compared (statistically) to the observed combination dose pair that gives the effect (in this illustration 50% of *E*_{max}). That is how a departure from simple additivity is determined. This determination is not

calculated directly from the steepness (slope) of the combinations dose-effect curve nor is it accomplished by a view of the shifted combination; it requires the actual observed dose combination that gives the 50% effect and the calculated combination dose from the additive isobole. I now mention the case in which the potency ratio is not constant, a situation that would apply when the individual log dose-effect curves are not parallel or when the individual drug maximum effects differ. In either case I proceed as above, i.e., I find the equivalent of dose a in terms of drug B but the equivalent is no longer aB/A . These situations lead to a nonlinear isobole of additivity as shown by Grabovsky and Tallarida (2004) for the different maxima case that is further described in the legend of Fig. 1III and is also discussed in Tallarida (2006) for two full agonists with a varying potency ratio. This possibility (nonlinearity) was recognized by Loewe (1927, 1928, 1953) but, because of his complex notation and the absence of a derivation, he did not make this fact explicit. Berenbaum (1989), in an extensive *Pharmacological Review* article, rejected the idea that the isobole can be curvilinear. To make his point Berenbaum provided a “proof” to support his claim. In this he constructed a “sham combination,” i.e., he considered a diluted form of drug A, called it drug B, and proceeded to show that a combination of drug A and this diluted form (drug B) lead to the linear form of Eq. 1. This is not a valid proof because drug A and its diluted form represent a situation in which the quantity of drug A needed to achieve the effect is always the same multiple of the amount of drug B that gives this effect when each drug acts as the sole agent. This means that the potency ratio is a constant, a situation that clearly leads to the linear isobole. So the Berenbaum proof is flawed in that it does not really address the case of a variable potency ratio. Loewe, who did not provide a mathematical proof, was nevertheless correct in recognizing that the additive isobole can be nonlinear. The recognition that the isobole can be nonlinear is crucial because the concavity of the set of observed points in a simply additive situation could be mistaken to be an indicator of synergism or antagonism depending on whether the observed dose pairs are below or above the isobole, respectively. The mathematical proof of the nonlinear isobole and the situation that leads to it are given in Grabovsky and Tallarida (2004) and are further described in Tallarida (2006). The concept of dose equivalence is fundamental in understanding Loewe’s approach to the isobole. Aside from its application in detecting synergism and subadditivity, the isobole is important because its derivation follows from a clear application of dose equivalence, and this is useful for illustrating other (related) ways to view synergism and antagonism. One of these other ways is from a view on the effect scale, which I discuss next.

Calculating the Combination Effect

In an article by Podolsky and Greene (2011) they point out some of the problems posed by regulatory agencies in their approval process for fixed-dose combinations based on the effect they produce and the required demonstration that they are “more effective” than the sum of their constituent parts. This aspect invites the question of how does one demonstrate the expected (additive) effect of a combination? This question is not discussed in any textbook of pharmacology that I am aware of. Yet, the determination of the expected effect is

important to compare that effect to the combination effect actually observed. A clear answer is afforded from the concept of dose equivalence, the same concept that I showed leads to the isobole. I illustrate this here for the situation of two drugs with constant potency ratio, but it is easily applicable to drugs with a varying potency ratio. In this illustration I will use fitted curves for drug A and B that are the familiar hyperbolic: $E = E_{\max} a/(a + A)$ and $E = E_{\max} b/(b + B)$. These have a constant potency ratio. To calculate the expected effect for the dose pair (a, b) I first transform dose a to its drug B equivalent, $b_{\text{eq}}(a) = a B/A$ for this case of constant potency ratio. Then the total dose, $a + b$, is equivalent to $b_{\text{eq}}(a) + b$, or $a B/A + b$. This total dose, when substituted in drug B’s dose-effect equation, gives the expected effect of the combination. Of course, it follows that any point (dose pair) on the isobole leads to the effect that defines that isobole. In other words, if the isobole is constructed for the 50% effect, this procedure leads to the combination effect = 50%. If the isobole is constructed for the 25% effect, this summing of dose $b + b_{\text{eq}}(a)$ leads the expected effect = 25%, etc. It is notable that the effect of the combination is not the sum of the effects produced by dose a alone and dose b alone. A numerical example is given in *Example*. The approach described here is easily generalized to three drugs, i.e., the drug B equivalent of a dose c of drug C could be determined and added to $b_{\text{eq}}(a)$ and b to get the total effective dose.

Dose-effect data for a two-drug combination can be plotted. If the data are placed on a three-dimensional Cartesian coordinate system with doses a and b in the plane, then the effect of (a, b) is plotted as the height above the plane at point (a, b) . All possible dose pairs plotted in this way (and smoothed) define a surface whose height above the planar point (a, b) is the expected (additive) effect of the dose combination. This surface therefore provides a view of how actual combination effects compare with the expected effects. Those effects above the surface indicate synergism and those on it indicate additivity, whereas effects below the surface indicate subadditivity. This approach is illustrated in Fig. 3, which uses data from a study of the combination of two endogenous vasoconstrictors (Lamarre and Tallarida, 2008).

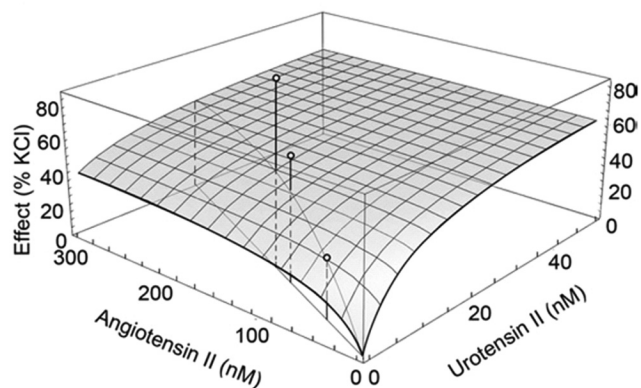


Fig. 3. Shown is a response surface for the effect of two vasoconstrictors on isolated aortic rings, where the effect is expressed as the percentage of tension produced by KCl. Three dose combinations in the fixed ratio 94:6 were tested and showed that the two highest doses produced effects above the additive surface. Based on Lamarre and Tallarida (2008) with permission.

The use of dose equivalence to get the combination additive effect is quite clear, and it is also the theoretical basis of the isobologram. But it is not the only approach that has been used. An alternate procedure for calculating the additive effect of a dose combination is given in the popular GraphPad program (GraphPad Software Inc., San Diego, CA). It is called the Bliss independence model of additivity (Bliss, 1939), and it uses only the individual drug effects without regard to the underlying dose-effect relations. In this method the fractional responses, FA of drug A and FB of drug B, are used to calculate the additive fractional effect, denoted Y, according to $Y = FA + FB - FA \times FB$. This formula (also called the Abbott formula of entomology) seems to have been derived from a mathematical model that is unrelated to potencies, but Bliss provided no detail, no derivation, and no extended discussion. The Bliss formula is inconsistent with the isobole as is readily demonstrated below (see *Example*). Whereas the method of dose equivalence described in this article uses only the potencies and efficacies of the constituent drugs, it should be noted that other approaches to study synergism have used intimate mechanisms based on enzyme kinetics and mass-action binding (Chou 2006).

Example. Suppose that drug A has $ED_{50} = 200$ mg and drug B has $ED_{50} = 20$ mg and these have a constant potency ratio, a situation that would apply when each obeys the hyperbolic relation, i.e., $E = 100 a/(a + 200)$ for drug A, and $E = 100 b/(b + 20)$ for drug B.

The linear isobole for the 50% effect is given by $a/200 + b/20 = 1$; therefore the point (100, 10) on the line is an additive combination for the 50% effect. But what does the Bliss formula give for this combination effect? The fractional response for $a = 100$ is clearly 0.3333 and that for $b = 10$ is also 0.3333. From the Bliss formula we get, $0.3333 + 0.3333 - (0.3333)(0.3333) = 0.5555$, or 55.55%. This is not 50%.

Another point on this 50% isobole is $a = 40$, $b = 16$. The Bliss formula for fractional effects Fa and Fb is $E_{ab} = Fa + Fb - Fa.Fb$ and this would lead to $Fa = 0.1667$ and $Fb = 0.4444$ so that $E_{ab} = 53.7\%$ and not 50%. Thus the Bliss formula is not consistent with the isobole.

Optimizing the Dose Combination

Synergism is not only a property of the two drugs; it also depends on the dose ratio as shown in other studies (Tallarida and Raffa, 1996). The isobologram shows this fact as the set of observed points in which some dose pairs (points) are below the isobole (synergistic), whereas others are close to or on the line (additive), and some others may be above the line (subadditive). These different points are indicative of different fixed-ratio dose combinations. Because an isobole can be determined for both the desired effect and any adverse effect of the drug combination, one should aim to locate dose combinations that are synergistic for the desired effect and subadditive for the adverse effect. This is an optimal strategy. In this regard I wonder, how do clinical investigators arrive at the dose ratios that they test in patients? In the absence of other information it would seem reasonable to choose the dose ratios by using observations from isobolar analysis in the preclinical studies that precede human testing. In that regard a recent article on a combination for treating breast cancer, a combination of pertuzumab, trastuzumab, and docetaxel (Baselga et al., 2012), is encouraging

based on survival rates, but the article gives no explanation for how the dose combination used was selected or whether other dose combinations were tried. Many studies of drug combinations have shown that different dose ratios give different degrees of synergism or antagonism. A properly conducted preclinical analysis with isoboles, or the dose-equivalent concept applied to the effect scale, can be a useful guide in selecting dose combinations in clinical trials. I have direct experience with this aspect from my collaborative work with Johnson & Johnson in contributing to the experimental preclinical design and analysis of that company's analgesic studies with tramadol and acetaminophen. In that effort we found a range of dose combinations that were synergistic and from which the company was awarded a United States patent (Raffa and Vaught, 1994). The company's clinical investigators were able to choose a dose ratio from among those that were synergistic, and that combination resulted in their product Ultracet. If a fixed-dose combination is optimal as defined previously, then the development of a product with the optimal dose ratio might overcome the objections and regulatory issues that are sometimes raised in developing such products.

Discussion: Summary

The isobole is based on the concept of dose equivalence. This is a clear application that uses the drugs' potencies in calculating the dose equivalent of one drug and adds it to the dose of the other to predict the combination's additive effect. This stands in contrast to methods derived from less clear mathematical models, many of which were described by Berenbaum (1989). When the potency ratio is constant, the isobole is linear. If the potency ratio is not constant, then the isobole is nonlinear. Regardless of its shape the isobole is equally useful in detecting synergism and antagonism, and therefore this well established method has enjoyed much usage. The same concept of dose equivalence that leads to the isobole also leads to a prediction of the combination's expected effect. An alternate approach, Bliss independence, is shown to be inconsistent with the isobole. Numerous preclinical studies of drug combinations have shown that synergism is not merely a property of the two drugs; it also depends on the dose ratio, a fact of potential importance in clinical testing.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Tallarida.

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Address correspondence to. Ronald J. Tallarida, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140. E-mail: ronald.tallarida@temple.edu
