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QUANTITATIVE ANALYSIS OF DOSE–EFFECT RELATIONSHIPS: THE COMBINED EFFECTS OF MULTIPLE DRUGS OR ENZYME INHIBITORS

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INTRODUCTION

The quantitative relationship between the dose or concentration of a given ligand and its effect is a characteristic and important descriptor of many biological systems varying in complexity from isolated enzymes (or binding proteins) to intact animals. This relationship has been analyzed in considerable detail for reversible inhibitors of enzymes. Such analyses have made assumptions on the mechanism of inhibition (competitive, noncompetitive, uncompetitive), and on the mechanism of the reaction for multi-substrate enzymes (sequential or ping-pong), and have required knowledge of kinetic constants (1–4). More recently, it has been possible to describe the behavior of such enzyme inhibitors by simple generalized equations that are independent of inhibitor or reaction mechanisms and do not require knowledge of conventional kinetic constants (i.e. K_m, K_i, V_{max}) (5–8).

Our understanding of dose–effect relationships in pharmacological systems has not advanced to the same level as those of enzyme systems. Many types of mathematical transformations have been proposed to linearize dose-effect plots, based on statistical or empirical assumptions, e.g. probit (9, 10), logit (11) or power-law functions (12). Although these methods often provide adequate linearizations of plots, the slopes and intercepts of such graphs are usually devoid of any fundamental meaning.

THE MEDIAN EFFECT PRINCIPLE

We demonstrate here the application of a single and generalized method for analyzing dose–effect relationships in enzymatic, cellular and whole animal systems. We also examine the problem of quantitating the effects of multiple inhibitors on such systems and provide definitions of summation of effects, and consequently of synergism and antagonism.

Since the proposed method of analysis is derived from generalized mass action considerations, we caution the reader that the analysis of dose–effect

data is concerned with basic mass-action characteristics rather than with proof of specific mechanisms. Nevertheless, it is convenient and intuitively attractive to analyze and normalize all types of dose–response results by a uniform method which is based on sound fundamental considerations that have physicochemical and biochemical validity in simpler systems. Our analysis is based on the median effect principle of the mass action law (5–8), and has already been shown to be simple to apply and useful in the analysis of complex biological systems (13).

The Median Effect Equation

The median effect equation (6, 8) states that:

$$f_a/f_u = (D/D_m)^m \qquad (1)$$

where D is the dose, f_a and f_u are the fractions of the system affected and unaffected, respectively, by the dose D, D_m is the dose required to produce the median effect (analogous to the more familiar IC_{50}, ED_{50}, or LD_{50} values), and m is a Hill-type coefficient signifying the sigmoidicity of the dose–effect curve, i.e., m = 1 for hyperbolic (first order or Michaelis–Menten) systems. Since by definition, $f_a + f_u = 1$, several useful alternative forms of equation 1 are:

$$f_a/(1 - f_a) = [(f_a)^{-1} - 1]^{-1} = [(f_u)^{-1} - 1)] = (D/D_m)^m$$

$$f_a = 1/[1 + (D_m/D)]^m$$

$$D = D_m[f_a/(1 - f_a)]^{1/m}$$

The median effect equation describes the behavior of many biological systems. It is, in fact, a generalized form of the enzyme kinetic relations of Michaelis–Menten (14) and Hill (15), the physical adsorption isotherm of Langmuir (16), the pH-ionization equation of Henderson and Hasselbalch (17), the equilibrium binding equation of Scatchard (18), and the pharmacological drug–receptor interaction (19). Furthermore, the median effect equation is directly applicable not only to primary ligands such as substrates, agonists, and activators, but also to secondary ligands such as inhibitors, antagonists, or environmental factors (5, 6).

When applied to the analysis of the inhibition of enzyme systems, the median effect equation can be used without knowledge of conventional kinetic constants (i.e. K_m, V_{max} or K_i) and irrespective of the mechanism of inhibition (i.e. competitive, noncompetitive or uncompetitive). Furthermore, it is valid for multisubstrate reactions irrespective of mechanism (sequential or ping-pong) (5–8).

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