Toxicology 402–403 (2018) 9–16

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

New insight into isobolographic analysis for combinations of a full and partial agonist: Curved isoboles

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ARTICLE INFO

Keywords:
Receptor theory
Partial agonist
Mixture toxicology
Synergism
Isoboles
Isobolographic analysis

ABSTRACT

Receptor ligands in mixtures may produce effects that are greater than the effect predicted from their individual dose-response curves. The historical basis for predicting the mixture effect is based on Loewe's concept and its mathematical formulation. This concept considers compounds with constant relative potencies (parallel dose-response curves) and leads to linear additive isoboles. These lines serve as references for distinguishing additive from nonadditive interactions according to the positions of the experimental data on or outside of the lines. In this paper, we applied a highly relevant two-state model for a description of the receptor-ligand interaction in the construction of the isobologram. In our model we consider partial agonists that have dose-response curve slopes differing from one. With this theoretical basis, we demonstrated that a combination of compounds with different efficacies leads to curved isoboles. This model should overwrite Tallarida's flawed assumption about isobolographic analysis of partial agonists and enhance our understanding of how the partial agonists contribute to the overall mixture effect.

1. Introduction

When two drugs cause their effects through the same receptor, their presence together usually produces a greater effect than the individual compounds alone. Over the past decade, many researchers tried to calculate and predict mixture effects of receptor ligand combinations. The combinations that correspond to the predicted effects that are based on the potencies of individual drugs are said to be additive. There are various models describing this kind of additivity, and the choice of a "correct" one is of great importance. Accordingly, the combined effects and their relation to the expected additivity are evaluated in terms of synergisms (effects greater than additive) or antagonisms (effects falling short of additivity). Choosing an inappropriate additivity model as a point of reference may result in the various mixture effects being erroneously determined as additive, synergistic, or antagonistic.

The model of concentration addition (CA) is broadly used as a null model in toxicology and pharmacology when compounds have similar mechanisms of their action. The concept of CA was introduced many years ago by Loewe and assumes that one compound can be substituted by another, proportional to their relative potencies. This situation can also be characterized by a simple equation:

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

Where a and b are the concentrations of the respective compounds in the mixture that produce a specific effect, and A and B are concentrations of the individual compounds that produce the same effect alone (Loewe and Muischnek, 1926; Loewe, 1953). The graphical interpretation of this equation is an isobologram, where each binary combination is plotted in a Cartesian coordinate system where the axes represent the individual drug doses. The straight line between the individual doses that produce the same specific effect is called the line of additivity (or isobole). The equation describing the line of additivity is derived only by rearrangement of the previous formulation.

$$b = -\frac{B}{A}a + B \tag{2}$$

Eq. (2) states that the slope (−B/A) and the intersection with the vertical axis (B) of the line of additivity could be simply calculated using the parameters of the individual dose-response curves. When the isobole for a specified effect has been determined, the dose pair of the combination that experimentally produces this effect may be plotted as a point that is below or above the line of additivity and thus indicate synergism (superadditivity) or antagonism (subadditivity), respectively. Isobolograms have been used in many studies of drug combinations (Bolan et al., 2002; Cichewicz and McCarthy, 2003; Fairbanks and Wilcox, 1999; Goldoni and Johansson, 2007; Grabovsky and

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https://doi.org/10.1016/j.tox.2018.04.004
Received 6 March 2018; Received in revised form 11 April 2018; Accepted 12 April 2018
Available online 13 April 2018
0300-483X/

Tallarida, 2004; Rossier et al., 2016; Tallarida, 2006; Wei and Roerig, 1998) and mathematical formulation displayed above has been used for the derivation of predictive mixture models (Howard and Webster, 2009; Safe, 1998).

The problem is that Loewe designed his concept for compounds with parallel dose-response curves with the same maximal effect. This concept assumes that the drugs have a constant relative potency. The aim of this article is to provide new insight into the analysis of drug combinations considering compounds with a variable relative potency, e.g., in experiments that employ full and partial agonists. This condition leads to a situation in which the isoboles are not straight lines. We began by examining the conventional situation of two full agonists and subsequently evaluated the situation in which one compound becomes a partial agonist. In our approach, we considered the two-state model of the receptor-ligand interaction that has a crucial role in the mechanism that describes how partial agonists trigger the effect (Ezechias and Cajthaml, 2018). In our approach, we do not consider receptors which could be in active state without any ligand (e.g., ion channels), and therefore, the basal effect (minima of dose-response curves) are always considered to be equal to 0.

2. Results and discussion

2.1. Combination of two full agonists

A situation when two full agonists have the same slope of their dose-response curves is displayed in Fig. 1a. In this situation, the compounds A and B have the same relative potencies for each effect level. Their respective EC_{50} values are 10 and 300 (e.g., μM). These concentrations represent half of the individual maximal effect and correspond with the inflection point of the resulting dose-response curve. The slope parameter (Hill coefficient) is set at the value 1.78 for both of the compounds. In its mathematic form, the respective Hill equations for compounds A and B are:

$$y_A = \frac{1}{1 + \left(\frac{1}{\frac{A}{10}}\right)^{1.78}} \tag{3}$$

$$y_B = \frac{1}{1 + \left(\frac{1}{\frac{B}{300}}\right)^{1.78}} \tag{4}$$

where y_A and y_B are the effects of the individual compounds; A and B are concentrations of the individual compounds. Because both of the compounds have the same maximum and slope, the dose-response curves are parallel, and any concentration of one compound could be substituted by the appropriate concentration of the second one to produce the same effect. This interpretation follows the main principle of Loewe concentration addition concept. In mathematic form:

$$\frac{1}{1 + \left(\frac{1}{\frac{A}{10}}\right)^{1.78}} = \frac{1}{1 + \left(\frac{1}{\frac{B}{300}}\right)^{1.78}} \tag{5}$$

and therefore

$$A = \frac{1}{30}B \tag{6}$$

If one compound could be substituted by the other, we can easily construct a model to calculate their combination effect.

$$y_{AB} = \frac{1}{1 + \left(\frac{1}{\frac{a}{10} + \frac{b}{300}}\right)^{1.78}} \tag{7}$$

where y_{AB} represents the combined mixture effect. Parameters a and b are the respective mixture concentrations. This equation is the simplest model that describes how to calculate a mixture effect, and it is identical with the Toxic Equivalency Factor approach (TEF, Safe, 1998).

Eq. (7) could easily be rearranged to a form of Loewe's equation for a chosen effect level y.

$$\frac{1}{y} = 1 + \left(\frac{1}{\frac{a}{10} + \frac{b}{300}}\right)^{1.78} \tag{8}$$

$$\sqrt[1.78]{\frac{1}{y} - 1} = \frac{1}{\frac{a}{10} + \frac{b}{300}} \tag{9}$$

$$\frac{1}{\sqrt[1.78]{\frac{1}{y} - 1}} = \frac{a}{10} + \frac{b}{300} \tag{10}$$

$$1 = \frac{a}{10\sqrt[1.78]{\frac{1}{y} - 1}} + \frac{b}{300\sqrt[1.78]{\frac{1}{y} - 1}} \tag{11}$$

Eq. (11) is the Loewe equation in which the concentrations of the individual acting compounds are represented by the respective inverse functions (Eqs. (3) and (4)). This conformity of Eq. (7) with Loewe's

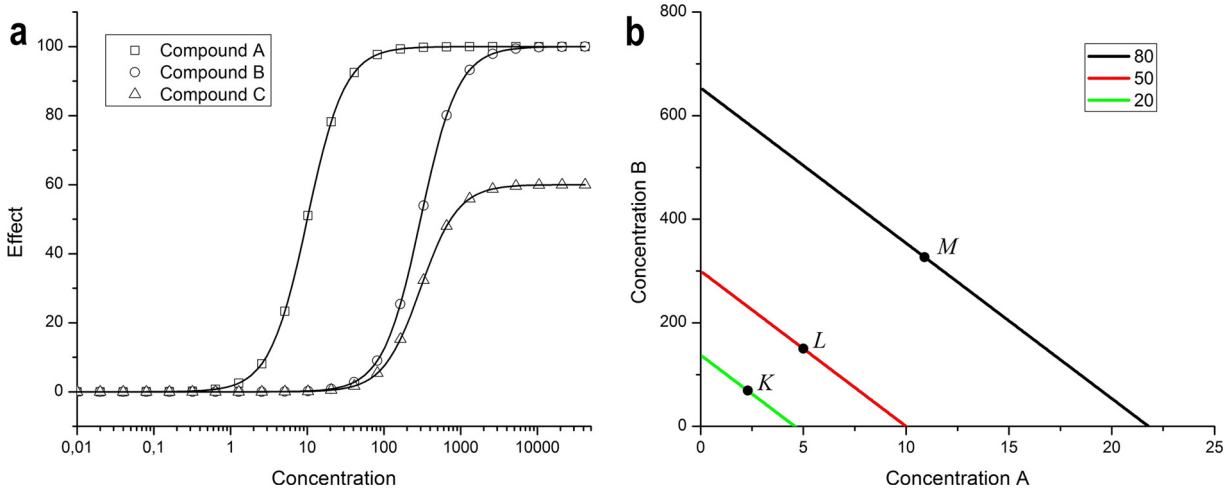

Fig. 1. Dose-response curves and resulted isoboles. (a) The dose-response curves for individually acting compounds A–C. (b) Isoboles for effect levels 20, 50 and 80 resulted from the combination of compounds A and B. Because both compounds are full agonists, these isoboles are straight and parallel, following the Loewe concentration additivity. Points K–M represents equi-effective dose-pairs for chosen effect levels.