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Current Methods for Quantifying Drug Synergism

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ABSTRACT

The effectiveness of drug combinations for treatment of a variety of complex diseases is well established. "Drug cocktail" treatments are often prescribed to improve the overall efficacy, decrease toxicity, alter pharmacodynamics, etc., in an overall treatment strategy. Specifically, if when combined, drugs interact in some way that causes the total effect to be greater than that predicted by their individual potencies, then drugs are considered synergistic. While there are established ways to quantify the impact of drug combinations clinically, it is an open challenge to quantitatively summarize a synergistic interaction. In this paper, we discuss an overview of the current statistical and mathematical methods for the study of drug combination effects, especially drug synergy quantification (where the interaction effect is not just detected, but quantified according to its magnitude). We first introduce two popular reference models for testing to null hypothesis of non-interaction for a combination, including the Bliss independence model and the Loewe additivity model. Then we discuss several methods for quantifying drug synergism. The advantages and disadvantages with these methods are also provided, and finally, we discuss important next directions in this area.

Keywords: Drug combinations, Synergy, Bliss, Loewe, Combination index

INTRODUCTION

For a variety of complex diseases, it is an accepted paradigm that drugs are given in combination [1]. A drug interaction is a situation in which another drug affects the activity of a drug when both are administered together. This action can be synergistic (when the drug's effect is increased) or antagonistic (when the drug's effect is decreased) [2]. The evaluation of combination effects between biological or chemical agents plays a significant role in pharmacology and biomedicine. Combination therapies, often referred to as "cocktail" therapies have revolutionized patient outcomes in diseases such as HIV [3], asthma [4], breast cancer [5,6], hypertension [7] and cancers such as melanoma [8]. The impact of chemical mixtures is also increasingly appreciated in the toxicology space as well, as people are not exposed to chemicals in isolation [9]. A recent review discusses the concept of synergy as used in a variety of fields [10].

The interaction of biologically or chemically active agents is often grouped into three categories: synergy, additivity (no interaction) and antagonism, based on the degree of departure of observed combination effects from the expected response without interaction [2,11]. Specifically, if drugs when combined interact with each other and cause a total effect that is greater than that predicted by their individual potencies, then this is considered a "synergistic drug" combination [12]. Such synergistic interactions can often reduce host toxicity and adverse side effects, given those doses of each drug in the combination are typically lower than that of single drugs to achieve desired efficacy. Additionally, such combination therapies can also reduce the development of drug resistance and other complications [13,14].

While the concept of synergy has been appreciated for a century, recent methods development and computational advances have allowed for new approaches for quantifying this phenomenon [15]. There are an emerging set of modeling approaches for quantifying synergism. These reference models have been developed based on distinctive biological and chemical assumptions. In addition, different methods are also developed to further detect and quantify synergistic effects specifically. It is typical in dose response assays to collect measures of drug response at multiple dose points. Such experiments can be expanded to assay both the individual drug responses from a pair or combination of

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drugs to include both the individual responses to the drug and then to the combinations. Such an experimental design provides the baseline information about response from a single drug, to compare to the response to synergistic combinations.

In this review, we provide an overview of the current statistical and mathematical methods for the study of drug combination effects, especially drug synergy quantification. We first introduce two popular reference models for the null hypothesis of non-interaction, which serve as the baseline to define synergy. Any deviation from the reference models will be regarded as synergy or antagonism. Subsequently, we discuss several statistical and mathematical approaches to quantify drug synergism. Finally, the common issues and opportunities with these methods are also provided. Although this paper mainly covers drug synergy, the concepts and methods mentioned in this review can be applied to other disciplines as well, such as toxicology and epidemiology.

TWO REFERENCE MODELS

To properly define synergy, it is of great importance to formulate a reference model for null hypothesis of non-interaction first, which suggests that the effects of drugs simply add up, not affecting each other (Additivity) [15]. Any deviation from the reference models will be regarded as synergy or antagonism, depending on the directions of departure. As shown in **Figure 1**, if the drug combination X and Y achieves the same response level with less dose than that of additive case (the reference model), the combination is said to by synergistic. Currently, there are two popular reference additivity models, Bliss independence model and the Loewe additivity model, which have different biological and chemical assumptions.

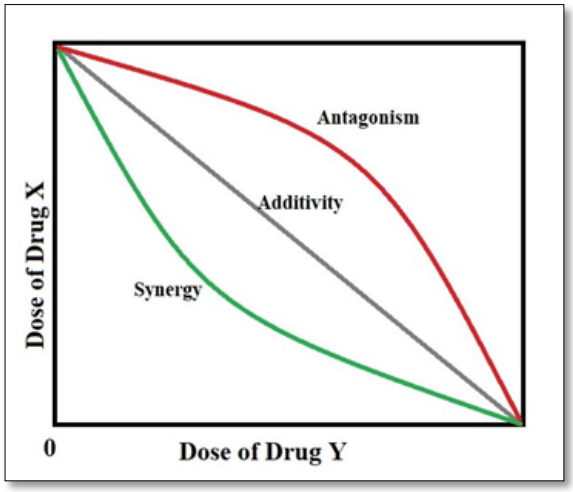

Figure 1. Comparison of additive, synergy and antagonism at the same response level. If the drug combination X and Y achieves the same response level with less dose than that of additive case (the reference model), the combination is said to by synergistic.

Bliss independence model

One of the oldest methods for quantifying synergy is the Bliss Independence model, dating back to the 1930s [16]. This model assumes that drugs do not interact with each other and elicit their responses independently [1]. According to independence probability theory, the expected response of drug combination R_c ($0 \le R_c \le 1$) can be written in terms of individual drug responses [16]:

$$R_c = R_1 + (1 - R_1) \times R_2 = R_1 + R_2 - R_1 \times R_2$$

Where drug 1 at dose y_1 produced a response R_1, drug 2 at dose y_2 produced a response R_2 and R_c is the expected response of drug combination 1 and 2 at dose y_1 and y_2, respectively. As the drug's effects R_1, R_2 and R_c are measured as the percentage of biological response, $0 \le R_1 \le 1$, $0 \le R_2 \le 1$ and $0 \le R_c \le 1$. Any observed response of drug combination greater than the expected response R_c can be interpreted as synergy and antagonism otherwise.

This null model is classically known in toxicology as "simple independent action" and is based on probabilistic independence. The paradigm is where there are two disjoint and independent causal pathways on which the two drugs act. The above equation can alternatively simply be rewritten as additivity in the logarithms of the two probabilities of nonresponse.

Loewe additivity model

An alternative null model is the additivity model, which assumes that drugs have similar modes of action on the same pathway [1,17]. In classical toxicology, this model is known as "simple similar action." It specifies that one drug's dose has the same effect on response as a scaling factor times the other drug's dose. To formulate this as specified in the Loewe additivity model, the dose-response relationship of individual drugs needs to be modeled first. Let the dose of drug 1=y_1 and the dose of drug 2=y_2. Then the Loewe additivity model can be expressed as the following equation [1,17]:

$$\frac{y_1}{Y_1} + \frac{y_2}{Y_2} = 1$$

Where Y_1 is the dose of drug 1 that achieves the same response level as the drug combination, y_1+y_2 and Y_2 is the dose of drug 2 that achieve the same response level as the drug combination. The left side of this equation is the widely used combination index. If a combination index is less than 1, synergy is declared. Similarly, a combination index greater than 1 can be interpreted as antagonism.

The major differences of the two reference models come from their underlying assumptions. The Bliss independence model assumes that drugs do not interact with each other and elicit their responses independently, whereas the Loewe additivity model assumes that drugs have similar modes of

action on the same pathway. Note that if the response is rare, the two formulations are asymptotically equivalent. In practice, the selection of one of those two to serve as the null model for assessing synergy and antagonism becomes largely a matter of personal preference [18]. To address the concerns raised in the two reference models, Yadav et al. [19] recently proposed a new reference model called zero interaction potency (ZIP). It evaluates drug interaction by comparing the change in dose response relationships between single drugs and their combinations. The results show that this new scoring method is able to keep the advantages of the two popular reference models mentioned above while overcome their limitations.

THE METHODS FOR QUANTIFYING DRUG SYNERGISM

Next, we will discuss methods for actually directly quantifying synergy. The methods are briefly introduced here, with references provided for a more detailed description of each approach.

Response surface

Response surface modeling is an approach to represent effects of drug combinations in three-dimensional plots where the doses of individual drugs are plotted as a horizontal x-y-plane, and the expected effect of drug combination is plotted on the z-axis, as shown in **Figure 2** [1]. Both the Bliss independence model and the Loewe additivity model can be used to calculate the expected effect of drug combination [1]. The experimental effect of a drug combination can then be plotted on this surface. Any departure from the 3D null surface is classified into synergism or antagonism, depending on the sign of the discrepancy as measured on the z-axis. [20,21].

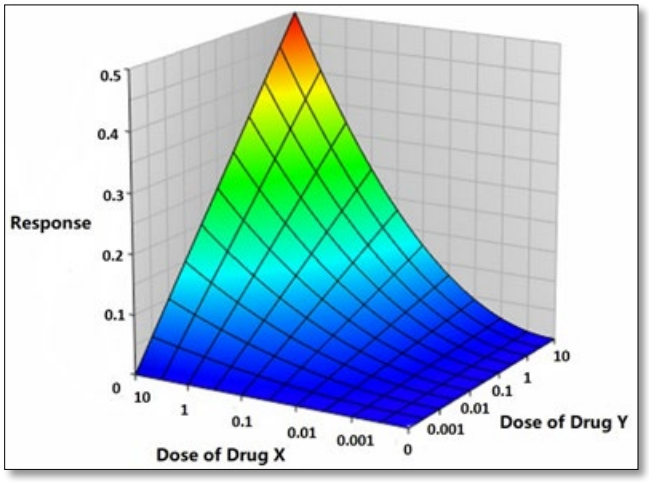

Figure 2. Example of response surface for two drugs, X and Y. The doses of individual drugs are plotted as a horizontal x-y-plane, and the expected effect (response) of drug combination is plotted on the z-axis.

Chou-Talalay method

The Chou-Talalay method is by far the most commonly used approach to quantify effects of drug combinations, especially synergistic interactions [15,21]. This method adopts the median-effect equation, which is derived from the unified theory mass-action law principle [9]. The median-effect equation is written below [21]:

$$\frac{f_a}{f_u} = \left(\frac{D}{D_m}\right)^m$$

Where f_a is the fraction affected by dose, f_u is the fraction unaffected by dose ($f_a+f_u=1$), D is dose of drugs given, D_m is the median-effect dose (e.g. IC50) and m is a parameter used to describe the shape of the dose-response curve.

The median-effect equation can be linearized by taking logarithm of both sides of the equation, as shown below:

$$\log\left(\frac{f_a}{f_u}\right) = m \times \log(D) - m \times \log(D_m)$$

Then the values of m and D_m can be estimated using linear regression. With this linear model, we can estimate the expected drug response values given specific drug doses, which will be used in the calculation of combination index (CI):

$$CI = \frac{D_1}{D_{x1}} + \frac{D_2}{D_{x2}}$$

Where D_1 and D_2 are the doses of two single drugs and D_{x1} and D_{x2} is the theoretical individual drug doses needed in order to achieve the same drug effect as the drug combination, which can be calculated based on the linear model mentioned above. CI<1 suggests synergism, CI=1 suggests additivity and CI>1 suggests antagonism [15,22].

One disadvantage of the Chou-Talalay method is that raw data must be preprocessed, including scaling the data and taking the log of a function of the scaled data [15].

MixLow method

More recently, Boik et al. [23] developed the MixLow method as an alternative to the Chou-Talalay method. The term MixLow means Mixed-effects Loewe, which has three components: a nonlinear mixed-effects model, the Loewe index and a method to calculate confidence intervals for the index. The MixLow method uses a nonlinear mixed effects model for estimating sigmoidal curve parameters from concentration-response data, and associated confidence intervals [23]. Compared with the Chou-Talalay method, the MixLow method produces more precise parameter estimation, and has improved coverage of confidence intervals. In addition, the use of a non-linear fixed-effects model in the MixLow method also eliminates the need for data preprocessing in the Chou-Talalay method [23].

Drug synergy quantification using a Bayesian approach

In 2010, Hennessey et al. proposed a Bayesian approach to dose-response assessment and synergy quantification. Briefly, they use a Bayesian hierarchical nonlinear regression model to explain the "variability between-experiments, variability within experiments, and variability in the observed responses of the controls" [24]. They first use Markov chain Monte Carlo (MCMC) to fit the model to the data. The second step is to carry out posterior inference on quantities of interest. Finally, they assess the presence of synergy while accounting for uncertainty using a modified version of Loewe additivity. Simulation results suggest that this method is more reliable in drug synergy estimation than the Chou-Talalay method, which often ignores important sources of variability and uncertainty that is generally the rule, instead of the exception in biology [24].

Summary of advantages and disadvantages of current methods (Table 1)

Table 1. Comparison of methods for quantifying drug synergism.

	Advantages	Disadvantages	Data type
Response Surface	Characterize the full concentration-response relationship	No formal quantification of the intensity of a synergistic interaction	Raw data
Chou-Talalay method	Linear regression can be applied	Raw data pre-processing and no statistical inference	Preprocessed raw data
MixLow method	More accurate estimation	Not easy to understand and use the method	Raw data
Bayesian approach	More accurate estimation	Potential complexity in Bayesian statistics	Raw data

CURRENT PROBLEMS AND FUTURE DEVELOPMENTS IN DRUG SYNERGY QUANTIFICATION

Drug combinations provide many advantages in the treatment of complex disease. The search for drug combinations has been widely recognized as one of the most important strategies for finding successful treatments of cancer and other diseases [15]. Although recent methods development has improved, there are still a number of open challenges and issues that need to be addressed.

First, there are still a number of challenges related to even defining synergy, much less quantifying it. In the current literature, the term synergy is not often clearly defined. Research papers usually use different reference models to quantify synergy in particular cases, which causes lack of comparability and confusion [18,25]. Thus, a standard reference framework should be developed to address the concerns raised in the current reference models and provide a clear definition of additivity, synergy and antagonism. Additionally, the standard framework should also be general enough to cover rare and specific cases so that researchers can use a universal method to quantify drug synergy. Our group has recently reviewed some of the challenges and differences in the terminology related to synergy [10].

Additionally, there are outstanding challenges in experimental design that need to be considered and advanced. One of the most important challenges of any study that will study synergy is the selection of dose and dose ratios in drug combination studies. The advantages of combination therapy not only depends on the properties of the drugs but also depend on the dose ratios [26,27]. Considering that two drugs combined at a given ratio are often treated as a new drug with its own dose-effect relationship in cells and tissues, we not only need to study whether a particular combination is synergistic, we also need to consider what dose ratio optimizes the synergistic interaction [26]. This is important in both experimental studies, and in clinical application.

Finally, we need to keep advancing more rigorous statistical methodology to interpret the variation in drug synergy quantification. Current methods quantify synergy, but do not ascribe a statistical confidence level with those estimates. Data from biological systems always carry experimental error and there is also inherent biological variation. However, the most commonly used combination indexes based on Bliss Independence and Loewe Additivity are often calculated without a suitable error assessment to measure the degree of uncertainty. The lack of a formal statistical framework in these approaches makes it difficult to interpret drug combination effects, especially for borderline cases.

CONCLUSION

In the current review, we discuss an overview of the current statistical and mathematical methods for the study of drug combination effects, especially drug synergy quantification.

We introduce two popular reference models for non-interaction of a combination, including the Bliss independence model and the Loewe additivity model. Then we discuss several methods for quantifying drug synergism. The advantages and disadvantages associated with these methods are also provided, and finally, we discuss current problems and future developments in drug synergy quantification.

Addressing these limitations represents an important methodological research direction. Recently there have been a number of new approaches to quantify dose response curves using machine learning methods, including evolutionary algorithms [28]. Such an approach could be extended to the drug combination effects as well.

Advances in the statistical methods will allow researchers to estimate the variability in biological or clinical experiments with sufficient accuracy and further improve the degree of confidence in drug synergy detection. Moreover, these advances will also benefit high-throughput drug combination screening greatly. The integration of automated screening techniques with robust statistical methods will facilitate the discovery of reliable synergistic drug interactions, ultimately improving the sensitivity and specificity of the screening process. Although we mainly discuss drug synergy here, these advances in statistical methods can be easily applied to other disciplines as well, such as environmental toxicology and epidemiology. For instance, we can detect the combination effects of multiple environmental chemicals for risk assessment purposes with a high degree of confidence.

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REFERENCES

1. Greco WR, Bravo G, Parsons JC (1995) The search for synergy: A critical review from a response surface perspective. Pharmacol Rev 47: 331-385.
2. Geary N (2013) Understanding synergy. Am J Physiol Endocrinol Metab304: 237-253.
3. Bartlett JA, DeMasi R, Quinn J, Moxham C, Rousseau F (2001) Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults. AIDS 15: 1369-1377.
4. Nelson HS (2001) Advair: Combination treatment with fluticasone propionate/salmeterol in the treatment of asthma. J Allerg Clin Immunol 107: 398-416.
5. Budman DR, Calabro A, Rosen L, Lesser M (2012) Identification of unique synergistic drug combinations associated with down expression of survivin in a preclinical breast cancer model system. Anticancer Drugs 23: 272-279.
6. Lee JH, Nan A (2012) Combination drug delivery approaches in metastatic breast cancer. J Drug Deliv 2012: 915375.
7. Glass G (2004) Cardiovascular combinations. Nat Rev Drug Discov 3: 731-732.
8. Held MA, Langdon CG, Platt JT, Graham-Steed T, Liu Z, et al. (2013) Genotype-selective combination therapies for melanoma identified by high-throughput drug screening. Cancer Discov 3: 52-67.
9. Carpenter DO, Arcaro K, Spink DC (2002) Understanding the human health effects of chemical mixtures. Environ Health Perspect 110: 25-42.
10. Roell KR, Reif DM, Motsinger-Reif AA (2017) An introduction to terminology and methodology of chemical synergy-perspectives from across disciplines. Front Pharmacol 8: 158.
11. Berthoud HR (2013) Synergy: A concept in search of a definition. Endocrinology 154: 3974-3977.
12. Cedergreen N (2014) Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PLoS One 9: e96580.
13. Greco WR, Faessel H, Levasseur L (1996) The search for cytotoxic synergy between anticancer agents: A case of Dorothy and the ruby slippers? J Natl Cancer Inst 88: 699-700.
14. Worthington RJ, Melander C (2013) Combination approaches to combat multidrug-resistant bacteria. Trends Biotechnol 31: 177-184.
15. Chou TC (2010) Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res 70: 440-446.
16. Bliss CI (1939) The toxicity of poisons applied jointly. Ann Appl Biol 26: 585-615.
17. Finney DJ, Uhbgcbie R (1964) Statistical method in biological assay. Hafner Pub. Co.
18. Tang J, Wennerberg K, Aittokallio T (2015) What is synergy? The Saariselka agreement revisited. Front Pharmacol 6: 181.
19. Yadav B, Wennerberg K, Aittokallio T, Tang J (2015) Searching for drug synergy in complex dose-response landscapes using an interaction potency model. Comput Struct Biotechnol J 13: 504-513.
20. Prichard MN, Shipman C Jr. (1990) A three-dimensional model to analyze drug-drug interactions. Antiviral Res 14: 181-205.
21. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: The combined effects of

multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22: 27-55.

22. Chou TC (2006) Theoretical basis, experimental design and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 58: 621-681.

23. Boik JC, Newman RA, Boik RJ (2008) Quantifying synergism/antagonism using non-linear mixed-effects modeling: A simulation study. Stat Med 27: 1040-1061.

24. Hennessey VG, Rosner GL, Bast RC Jr., Chen MY (2010) A Bayesian approach to dose-response assessment and synergy and its application to *in vitro* dose-response studies. Biometrics 66: 1275-1283.

25. Jia J, Zhu F, Ma X, Cao Z, Cao ZW, et al. (2009) Mechanisms of drug combinations: Interaction and network perspectives. Nat Rev Drug Discov 8: 111-128.

26. Keith CT, Borisy AA, Stockwell BR (2005) Multicomponent therapeutics for networked systems. Nat Rev Drug Discov 4: 71-78.

27. Straetemans R, O'Brien T, Wouters L, Van Dun J, Janicot M, et al. (2005) Design and analysis of drug combination experiments. Biom J 47: 299-308.

28. Beam AL, Motsinger-Reif AA (2011) Optimization of nonlinear dose- and concentration-response models utilizing evolutionary computation. Dose Response 9: 387-409.