JOURNAL OF VIROLOGY, Oct. 2002, p. 10577–10578 Vol. 76, No. 20
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.20.10577–10578.2002

Letter to the Editor
Synergy Determination Issues

A recent paper by F. Verrier and coworkers presented the combination of antibodies against a human immunodeficiency virus (HIV) type 1 isolate (8). In the paper was described a "new mathematical treatment" of determining synergism, additive effect, or antagonism. Its verbatim reasoning used symbols but without any mathematical derivations, since it is claimed to be "model free." It is not possible to find the origin of these equations without changing symbols of equations previously published by others. It further discussed Chou and Talalay's equations and method (7) and indicated their deficiencies. As a co-originator of the Chou-Talalay combination index method, I would like to respond to the issues raised by Verrier et al. and to point out that the comments of Verrier et al., in many parts, are erroneous or confusing.

(i) A close examination of their paper indicates that the new method of Verrier et al. is the same as the Webb method (i.e., the fractional product method) published nearly 40 years ago (9).

(ii) Chou and Talalay have actually derived the fractional product equation based on the mass-action law principle and proved that the fractional product method has the following limitations (1, 3, 4, 7): (a) It is valid only for pure, mutually nonexclusive conditions (e.g., no conformational changes or no allosteric effects). (b) It is valid only when the dose-effect relationships show exact hyperbolic curves (i.e., $m = 1$) but is not valid for sigmoidal curves (i.e., $m \neq 1$). (In reality, for most biological systems, the value of m is $\neq 1$.) (c) It is not consistent with the classic isobologram. (Chou and Talalay had to present the nonclassic conservative isobologram to describe the nonexclusive case.) (d) It takes into account the potency but totally ignores the shape of the dose-effect curves of each drug involved in the combination. (e) It leads to underestimation of synergism or overestimation of antagonism when compared with the classic isobologram method.

Chou and Talalay mathematically derived over 200 equations and have considered various conditions (e.g., number of reactants and products, reaction mechanisms and sequences, type of inhibition, exclusivity of inhibition, etc.) before publishing their generalization (1, 3, 4, 5, 7). To date, the median-effect equation of Chou and the combination index method of Chou and Talalay have been cited in over 1,750 biomedical scientific papers worldwide. For reference 7 alone, there have been over 813 citations since 1984 (based on Web of Science statistics; www.isiglobalnet.com). Although some of the comments on the Chou and Talalay method by Verrier et al. are correct, the following description and comments are inaccurate:

(i) "The theory of Chou and Talalay is based on enzyme kinetics": the enzyme kinetic models used by Chou and Chou-Talalay are entirely based on the mass-action law principle. Enzyme kinetics is only used as the model or tool (1, 3, 4, 7). Mass-action law is the fundamental rule of the physicochemical world. The statistical approach has been used for drug combination studies for more than 60 years and has not yet shown general acceptance. While statistics are useful for probability, correlation, variance, and significance, they do not form the basis for the dose-effect relationship mechanisms of ligands, reactants, or chemicals.

(ii) "This [the Chou-Talalay method] requires that both Abs [drugs] used in a combination be capable of neutralizing the virus used [efficacious] in the experiment": if one of the components in the combination has no effect, then it is not a drug and synergism or antagonism is irrelevant. This issue has been clearly defined by Chou et al. as potentiation-enhancement or inhibition-suppression. In this simple arithmetical situation, percent potentiation, fold enhancement, etc., will suffice for its quantitation (2, 6, 7).

(iii) ". . . therefore they are not mutually exclusive in their ability to bind": the mutually exclusive and mutually nonexclusive combinations are the two extreme cases used in Chou-Talalay's theoretical derivations since the 1970s (1, 3, 4). Following years of application in experimental systems, it has been concluded that if a unified method is to be used in the absence of exact knowledge of exclusivity, the choice will be exactly consistent with the classic isobologram, which is the exclusive case (2, 6). The general isobologram equation for two or more drug combinations was derived by Chou and Talalay in 1984 (7).

REFERENCES

1. **Chou, T.-C.** 1974. Relationships between inhibition constants and fractional inhibitions in enzyme-catalyzed reactions with different numbers of reactants, different reactions mechanisms, and different types of mechanisms of inhibition. Mol. Pharmacol. **10:**235–247.
2. **Chou, T.-C.** 1991. The median-effect principle and the combination index for quantitation of synergism and antagonism, p. 61–102. *In* T.-C. Chou and D. C. Rideout (ed.), Synergism and antagonism in chemotherapy. Academic Press, San Diego, Calif.
3. **Chou, T.-C., and P. Talalay.** 1977. A simple generalized equation for the analysis of multiple inhibitions of Michaelis-Menten kinetic systems. J. Biol. Chem. **252:**6438–6442.
4. **Chou, T.-C., and P. Talalay.** 1981. Generalized equations for the analysis of inhibitors of Michaelis-Menten and higher order kinetic systems with two or more mutually exclusive and nonexclusive inhibitors. Eur. J. Biochem. **115:** 207–216.
5. **Chou, T.-C., and P. Talalay.** 1987. Applications of the median-effect principle for the assessment of low-dose risk of carcinogens and for the quantitation of synergism and antagonism of chemotherapeutic agents, p. 37–64. *In* K. R. Harrap and T.A. Connors (ed.), New avenues in developmental cancer chemotherapy, Bristol-Myers Symposium series. Academic Press, New York, N.Y.
6. **Chou, T.-C., D. Rideout, J. Chou, and J. R. Bertino.** 1991. Chemotherapeutic synergism, potentiation and antagonism, p. 371–379. *In* R. Dulbecco (ed.), Encyclopedia of human biology, vol. 2. Academic Press, San Diego, Calif.
7. **Chou, T.-C., and P. Talalay.** 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs on enzyme inhibitors. Adv. Enzyme Regul. **22:**27–55.
8. **Verrier, F., A. Nádas, M. K. Gorny, and S. Zolla-Pazner.** 2001. Additive effects characterize the interaction of antibodies involved in neutralization of the primary dualtropic human immunodeficient virus type 1 isolate 89.6. J. Virol. **75:**9177–9186.
9. **Webb, J. L.** 1961. Effect of more than one inhibitor, p. 66 and 488. *In* Enzymes and metabolic inhibitors, vol. 1. Academic Press, New York, N.Y.

Ting-Chao Chou
Memorial Sloan-Kettering Cancer Center
1275 York Ave.
New York, NY 10021

Phone: (212) 639-7480
Fax: (212) 794-4342
E-mail: chout@mskcc.org

Authors' Reply

We agree with a number of assertions in Dr. Chou's letter regarding the method we used in our recent paper (3) to quantify synergistic neutralization of HIV by pairs of monoclonal antibodies. However, we disagree with much of what the letter contains; in particular, we reject the assertion that we made certain erroneous comments in our paper.

We are criticized for not presenting mathematical derivations that lead to our "model-free" approach. Indeed, we do not model the dose-response relationship between antibody concentration and neutralizing effect as a mathematical curve. In our case, knowing the response at various doses carries no information about the response at some other dose not among these. This empirical approach differs from the Chou-Talalay approach, which, using different language and reasoning, does arrive at a dose-response model which is well known to statisticians as the logistic regression model. In such a model, the knowledge of the response at a few points determines the response at all doses.

In response to points i and ii in Dr. Chou's letter, we state the following: the method we employed is the same as the Webb method and we are glad to acknowledge priority. The method is a simple application of a basic probability concept: namely, the probability that at least one of two independent events occurs (4). Dr. Chou states that the Webb fractional product method which we apply is valid only for mutually nonexclusive conditions. We accept this, but point out that we have assumed that because the antibodies of interest have different epitopes, we are dealing with mutually nonexclusive conditions; therefore, the method we use is applicable. Dr. Chou then goes on to state that this method "is valid only when the dose-effect relationships show exact hyperbolic curves," "is not consistent with the classic isobologram," "ignores the shape of the dose-effect curves," and the results differ from those which would be obtained by the classic isobologram method. These comments, however, are not meaningful in the context of our work, since we do not model a dose-response curve nor do we make explicit use of the properties of isobolograms.

We also reject Dr. Chou's reasoning that his method should have been used because he and his colleagues have derived a large number of equations and their work has been cited in many papers. We agree that the method of Chou and Talalay has been extremely useful (in fact, we have used it in relevant work that we published previously (1, 2). Nevertheless, Dr. Chou's reasoning that this alone supports its application to our studies belongs to the category of argumentum ad populum and for this reason, it is without technical merit.

Further, the letter lists three quotations from our paper with the claim that they are inaccurate. We disagree with this characterization in all three cases:

(i) We described the application of the method of Chou and Talalay to the field of enzyme kinetics and agree that it is based on the mass-action law principle. We reiterate, however, that our use of "statistics . . . probability, correlation, variance, and significance" is applicable to the problem we describe, which does not model dose-effect relationships.

(ii) Dr. Chou states that synergy (or antagonism) cannot occur if one of the two reagents being tested has no effect by itself. However, it is well established that the mixture of one "nonreactive" reagent and another "reactive" reagent can lead to a greater (or different) effect than that seen with either reagent alone. (This is the case, for example, with an enzyme and its substrate.) The Chou and Talalay method does not apply to this situation. It was for this reason that their approach could not be applied to the analysis of our work and that we developed and applied the method described in our paper.

(iii) Dr. Chou reiterates his choice of an isobologram method. As we state above, and as described in detail in the Appendix of our paper, the conditions of our experiments (the study of the interaction of one neutralizing and one nonneutralizing monoclonal antibody) preclude the use of the Chou and Talalay method. We justify and apply a model-free approach based on a simple, probabilistic method for analyzing the data.

REFERENCES

1. **Buchbinder, A., S. Karwowska, M. K. Gorny, S. T. Burda, and S. Zolla-Pazner.** 1992. Synergy between human monoclonal antibodies to HIV extends their effective biologic activity against homologous and divergent strains. AIDS Res. Hum. Retrovir. **8:**425–427.
2. **Laal, S., S. Burda, M. K. Gorny, S. Karwowska, A. Buchbinder, and S. Zolla-Pazner.** 1994. Synergistic neutralization of human immunodeficiency virus type 1 combinations of human monoclonal antibodies. J. Virol. **68:**4001–4008.
3. **Verrier, F., A. Nádas, M. K. Gorny, and S. Zolla-Pazner.** 2001. Additive effects characterize the interaction of antibodies involved in neutralization of the primary dualtropic human immunodeficiency virus type 1 isolate 89.6. J. Virol. **75:**9177–9186.
4. **Webb, J. L.** 1961. Effect of more than one inhibitor, p. 66 and 488. *In* Enzymes and metabolic inhibitors, vol. 1. Academic Press, New York, N.Y.

Arthur Nádas
The Institute for Environmental Medicine
New York University School of Medicine
New York, NY 10016

Susan Zolla-Pazner*
Veterans Affairs Medical Center
Room 18124N
423 E. 23rd St.
New York, NY 10010-5050

*Phone: (212) 263-6769
Fax: (212) 951-6321
E-mail: zollas01@endeavor.med.nyu.edu