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Antiviral Research 25 (1994) 1–11

Antiviral Research

# Multiple drug effect analysis with confidence interval

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Received 9 December 1992; accepted 22 March 1994

**Abstract**

The development of mathematical models for determining antagonism, synergy or additivism for drug combinations is becoming increasingly important in medical research and medicine. This paper describes improvements in the Chou and Talalay method which provides for the first time confidence intervals for the combination index. The Monte Carlo technique was used to calculate the confidence intervals and to obtain the new parameters which are needed for the modified interaction diagnosis. This new method can be adapted to current as well as future formulae used to evaluate drug combinations. Programs written in Microsoft and Visual Basic were developed for use on commonly available personal computers which we call Combostat®.

*Key words:* Drug interaction; Monte Carlo simulation; Combination index; Confidence interval

Abbreviations: $\overline{CI}$, mean value of the combination index determined by the Monte Carlo technique; $CI$, combination index; $CI$B, confidence interval boundary; CCSI, correlation coefficient between the slope and $y$-intercept of the median-effect plot; $D_m$, median effective dose of a drug applied alone; $d_m$, median effective dose of a drug in a mixture; $\delta_m$, relative error of the slope; $\delta_{y\text{-int}}$, relative error of the $y$-intercept; $\delta_\Delta$, relative error of the combination index; $E$(D), mean value of the dose; $F_a$, fraction affected; $m$, slope of the median-effect plot; $N$, number of statistical computations; $r$, linear correlation coefficient; S.D. or $\sigma$, standard deviation; S.E.$_m$, standard error of the slope; S.E.$_{y\text{-int}}$, standard error of $y$-intercept; $y$-int, median-effect curve intercept with the $y$-axis; $X$, $X'$, and $Y$, random numbers; $\Sigma$, parameter which characterizes the proximity of the mean value $\overline{CI}$ to the corresponding value of $CI$. It is equal to $(\overline{CI}\text{-}CI)/CI$; $\Delta$, magnitude of the confidence interval.

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*SSDI* 0166-3542(94)00033-5

## 1. Introduction

Combined chemotherapeutic approaches have been used extensively to reduce drug toxicity, prevent or delay the development of resistant organisms or cells, and to obtain a greater effect than with one active drug alone (Schinazi, 1991). Various analytical models have been developed to determine the type of interaction obtained with compounds in combination (Chou and Talalay, 1984; Greco et al., 1990; Kageyama et al., 1992; Lam et al., 1991; Prichard and Shipman, 1990; Schinazi, 1991; Sühnel, 1990). All these methods, including recent methods such as the Prichard-Shipman or the COMBO method have advantages and disadvantages (Berenbaum, 1988; Chou, 1991; Greco et al., 1990; Kageyama et al., 1992; Lam et al., 1991; Prichard and Shipman, 1990; Rideout and Chou, 1991; Schinazi, 1991; Sühnel, 1990; Syracuse and Greco, 1986). The median-effect principle and combination index method for quantitation of synergism and antagonism (also known as the Chou and Talalay method) are the most widely used techniques for studying drug interactions, especially in antiviral and anti-cancer research (Chou, 1991; Chou and Talalay, 1984; Rideout and Chou, 1991; Schinazi, 1991). The popularity of this method rests primarily with the simplicity of the experimental design; a relatively small number of data are required for determining the type of interaction and its computer program is user-friendly. The theoretical background and experimental design of this method was originally developed by Chou and Talalay (1984). Specific examples of combinations of various compounds using the Chou and Talalay method have also been published (Eriksson et al., 1989; Johnson et al., 1991; Kong et al., 1992; Rideout and Chou, 1991; Schinazi, 1991; Schinazi et al., 1986, 1990; Tilley et al., 1992; Vogt et al., 1987).

In spite of the wide-spread use of this technique and a long history of studies performed using the Chou and Talalay method, there continues to be deficiencies with this method. These include: (1) Lack of confidence interval for the Combination Index ($CI$) in the multiple drug-effect analysis; (2) The interaction diagnosis never indicates summation (additivism), only synergism or antagonism can be determined qualitatively; (3) The standard errors for the median-effect parameters, provided by regression analysis in the combination index method, are not used for $CI$ calculation and interaction diagnosis; (4) There is no statistical criteria for comparing the accuracy of results obtained from different combination experiments with the same agents; and (5) The $CI$ method does not indicate the situation when the interaction diagnosis cannot be made because of lack of statistically acceptable data.

Thus, the purpose of this work was to develop the statistical approach for the confidence interval for the $CI$ determination and to provide new parameters which would improve the analysis. We are also aware that there is an ongoing controversy regarding the Chou and Talalay equations for the mutually non-exclusive case. Taking that into account, we have developed a general approach which can be readily modified once consensus among scientists working in this area is reached. For the mutually exclusive case, there is consensus and no modification to the Chou and Talalay equation is needed.

## 2. Materials and methods

### 2.1. Method for calculation of the confidence interval for the CI

In order to conduct the statistical analysis on the median-effect principle and *CI* method we used Monte Carlo technique or method of Statistical Trials (Buslenko et al., 1966; Bratley et al., 1983). The main thrust of this approach consists of the following. Assume that someone had infinite resources and repeated the combination experiment several times. This individual would be able to obtain several values for the *CI* for every fraction affected ($F_a$) on the $F_a$-*CI* plot and would then be able to accurately estimate the error for *CI*. However, this approach would be inefficient in terms of time, cost, and efforts.

Another way to accomplish this goal is to use a probabilistic model. Instead of repeating the real experiment several times, one can create a probabilistic model and repeat a numerical experiment. The Monte Carlo Technique can be appropriately used for this purpose as it allows the simulation of any processes influenced by random factors.

1. According to median-effect method (Chou and Talalay, 1984), the *CI* is calculated by using the following numerical scheme: For each drug alone and for their combination, the linear portion of the dose-effect curves and the unweighted linear regression analysis (Neter et al. 1985) are used to calculate:
   - the slope of the median-effect curve ($m$), and its standard error ($S.E._m$);
   - the $y$-intercept ($y$-int) and its standard error ($S.E._{y\text{-int}}$);
   - the dose that is required to produce a median-effect ($D_m$). $D_m$ is determined as $D_m = 10^{-(y\text{-int}/m)}$;
   - the linear correlation coefficient of the median effect plot ($r$).

   Then one calculates the *CI* for different levels of fractional effect ($F_a$) by the formula:

$$CI = \frac{d_1}{D_1} + \frac{d_2}{D_2} + \alpha\frac{d_1 d_2}{D_1 D_2}. \tag{1}$$

   where $d_1$ and $d_2$ are doses of agents 1 and 2 in a mixture to produce a given effect level, $D_1$ and $D_2$ are the doses of the agents required to produce the same effect level when applied alone, $a = 0$ for the mutually exclusive case and $a = 1$ for the mutually non-exclusive agents. Note that for the mixture the following equations are fulfilled: $d_1 + d_2 = d_{1,2}$, $d_1 = d_{1,2} \times A/(A + B)$ and $d_2 = d_{1,2} \times B/(A + B)$ where $A/B = d_1/d_2$, and $d_{1,2} = [F_a/(1 - F_a)]^{1/m_{1,2}} \times d_{m1,2}$ where $d_{m1,2}$ is the dose that is required to produce a median-effect for the combination.
2. The statistical variation of the dose-effect data results in the median-effect parameters errors ($S.E._m$ and $S.E._{y\text{-int}}$). The simulation procedure should model two effects: the random slope ($\tilde{m}$) of the median effect curve on the $F_a$-D plot and random displacement of this curve intercept with the $Y$-axis ($\tilde{y}$-int). These two effects might

be simulated by using the two random numbers $X$ and $Y$ with average values equal to 0, and the variances equal 1 and based on the formulas:

$$\tilde{m} = m + X \times \text{S.E.}_{\text{m}} \quad \text{and} \quad \tilde{y}\text{-int} = y\text{-int} + Y \times \text{S.E.}_{\text{y-int}}. \tag{2}$$

However, besides the mean values $m$ and $y$-int and standard errors $\text{S.E.}_{\text{m}}$ and $\text{S.E.}_{\text{y-int}}$, the simulation procedure should include a mutual correlation between the slope and $Y$-intercept of the median-effect plot. According to (Neter et al. 1985), the corresponding correlation coefficient is equal:

$$\text{CCSI} = -E(\text{D})\frac{\text{S.E.}_{m}}{\text{S.E.}_{y\text{-int}}}, \tag{3}$$

where $E(\text{D})$ is the mean value of the dose. Thus, the mean value of the dose $E(\text{D})$ and the correlation between the slope and $Y$-intercept (CCSI) for each drug alone and for their combination in addition to the median-effect parameters listed above in item 1, should be calculated. Finally, in order to generate two random numbers ($X$ and $Y$) with a specific correlation coefficient (CCSI) determined by Eqn. 3, a special procedure expressed by the following formula (Bratley et al., 1983) should be used.

$$Y = \text{CCSI} \times X + (1 - \text{CCSI}^2)^{1/2} \times X', \tag{4}$$

where $X$ and $X'$ are the statistically independent random numbers with the average values equal to 0 and variances equal 1.

3. When the random values $\tilde{m}$ and $\tilde{y}$-int are generated, Eqn. 1 is used to calculate the $CI$ for each level of $F_a$. By repeating this process $N$ times the statistical data for the $CI$ for each level of $F_a$ can be obtained. After that one can calculate the mean value of the $CI(\overline{CI})$ and its standard deviation (S.D.). The proximity of the $\overline{CI}$ to the correspondent value of $CI$ determined by the parameter:

$$\sum = \frac{\overline{CI} - CI}{CI}, \tag{5}$$

which characterizes the accuracy of the modeling. The 95% confidence interval at each level of $F_a$ is calculated by the formula: $CI \pm (1.96 \times \text{S.D.})$, where the values $CI + (1.96 \times \text{S.D.})$ and $CI - (1.96 \times \text{S.D.})$ correspond to the upper and lower boundaries of the confidence interval, respectively.

It should be noted that such simulation technique allows the application of different assumptions for the probability distribution for the median-effect parameters errors. Nevertheless, in practice it requires that the distribution be specified. Assuming that the slope and the intercept errors are distributed according to Gaussian distribution law, we used the subroutine TRPNRM derived from the method of Ahrens and Dieter (Bratley et al., 1983) to generate the normal random numbers $X$ and $X'$. However, the calculations performed under two different assumptions for the probability distribution law (Gaussian and uniform) show that the relative variations of the magnitude of the confidence interval is not greater than 10%. Note also that the simulation procedure described above is universal. It might be equally applied to equation (1) which has been developed by Chou and Talalay or to alternative formulae which were proposed later by Syracuse and Greco (1986) and Lam et al. (1991).

### 2.2. The interaction diagnosis with confidence interval and statistical criteria

The interaction diagnosis in the *CI* method is based on the comparison of the *CI* value with 1; when $CI < 1$, synergism of effects is indicated, the value $CI > 1$ corresponds to antagonism, and $CI = 1$ indicates summation (Chou and Talalay, 1984). As the plot of *CI* vs. $F_a$ can either intersect the line $CI = 1$ at one point or not intersect it at all, the summation of effects in the *CI* method usually is not indicated.

Taking into account the above determined confidence interval, the interaction diagnosis can be modified by the following way. Consider that an antagonistic effect takes place when the condition $CI - (1.96 \times \text{S.D.}) > 1$ is fulfilled. The condition $CI + (1.96 \times \text{S.D.}) < 1$ will indicate synergism and two conditions, namely $CI + (1.96 \times \text{S.D.}) > 1$ and $CI - (1.96 \times \text{S.D.}) < 1$ will correspond to summation. As the magnitude of confidence interval ($\Delta = 1.96 \times \text{S.D.}$) is a finite value, the modified interaction diagnosis may indicate summation.

If the magnitude of confidence interval $\Delta \gg 1$ or the ratio $\delta_\Delta = \Delta/CI \gg 1$, it is difficult to make any diagnosis since this indicates that the experimental results are not adequate for statistical analysis. Since the ratio of $\delta_\Delta = \Delta/CI$ does not depend on the absolute values of $\Delta$ and *CI* and characterizes the statistical accuracy of the results (the relative error for the *CI*) one can use the *CI* error parameter ($\delta_\Delta$) for comparison of results of different experiments.

In general, the above *CI* error parameter, $\delta_\Delta$ depends on a number of factors:

$$\delta_\Delta = \text{f}\left(F_{\text{ai}},\ m_{\text{i}},\ \text{S.E.}_{\text{mi}},\ y\text{-int}_{\text{i}},\ \text{S.E.}_{y\text{-int}_{\text{i}}},\ r_{\text{i}},\ \text{CCSI}_{\text{i}}\right). \tag{6}$$

where *i* represents parameters for drug one, drug two or the combination of drugs. In the next section, we will determine which of the above parameters influences $\delta_\Delta$ value the most, and how this value depends on the variation of the most important parameters in Eqn. 6.

### 2.3. Examples of application and variations of confidence interval

In order to demonstrate the advantages of the new technique, we first applied it to analyze dose-effect data published by Chou and Talalay (1984). The results obtained were compared with those determined by the *CI* method without confidence interval.

The dose effect data points with the median effect parameters and the correlation coefficients between the slope and *y*-intercept for two examples taken from Chou and Talalay (1984) are shown in Tables 1 and 2. In Tables 3 and 4, the following parameters were determined using the new technique: *CI*, its mean value ($\overline{CI}$) determined by the Monte Carlo Technique, upper $[CI + (1.96 \times \text{S.D.})]$ and lower $[CI - (1.96 \times \text{S.D.})]$ boundaries of the confidence interval (*CI*B), the magnitude of confidence interval $\Delta = 1.96 \times \text{S.D.}$ and the *CI* $\delta_\Delta$. *CI* values in Tables 3 and 4 coincide with the corresponding results obtained by Chou and Talalay (1984). Note that calculations were made using $N = 500$, where *N* is the number of statistical computations.

The results of interaction diagnosis with and without confidence interval are also presented in Tables 3 and 4. As noted in the tables, the results of the two diagnoses do not agree with each other. From the data presented in Tables 3 and 4, it follows that the

Table 1
Inhibition of horse liver alcohol dehydrogenase by *ADP*-ribose and *ADP*

| Compound | | Fractional inhibition ($F_a$) | Median-effect parameters |
|---|---|---|---|
| ADP-ribose | ADP | | |
| 95 | | 0.389 | |
| 190 | | 0.535 | *Y*-int: $-2.12 \pm 0.07$ |
| 285 | | 0.639 | *m*: $0.96 \pm 0.03$ |
| 380 | | 0.707 | *r*: 0.998 |
| 475 | | 0.748 | CCSI: −0.995 |
| | 0.5 | 0.224 | |
| | 1.0 | 0.371 | *Y*-int: $-0.23 \pm 0.004$ |
| | 1.5 | 0.468 | *m*: $1.04 \pm 0.02$ |
| | 2.0 | 0.555 | *r*: 0.999 |
| | 2.5 | 0.605 | CCSI: −0.422 |
| Mixture (180:1) | | | |
| 95.5 | | 0.472 | |
| 191 | | 0.637 | *Y*-int: $-2.04 \pm 0.04$ |
| 286.5 | | 0.734 | *m*: $1.00 \pm 0.015$ |
| 382 | | 0.781 | *r*: 0.999 |
| 477.5 | | 0.817 | CCSI: −0.995 |

Table 2
Inhibition of horse liver alcohol dehydrogenase by *o*-phenanthroline and *o*-phenanthroline and *ADP*

| Compound | | Fractional inhibition ($F_a$) | Median-effect parameters |
|---|---|---|---|
| *o*-phenanthroline | ADP | | |
| 8.7 | | 0.132 | |
| 17.4 | | 0.267 | *Y*-int: $-2.04 \pm 0.06$ |
| 26.1 | | 0.411 | *m*: $1.3 \pm 0.04$ |
| 34.8 | | 0.476 | *r*: 0.998 |
| 43.5 | | 0.548 | CCSI: −0.98 |
| | 0.5 | 0.175 | |
| | 1.0 | 0.400 | *Y*-int: $-0.26 \pm 0.03$ |
| | 1.5 | 0.492 | *m*: $1.18 \pm 0.127$ |
| | 2.0 | 0.542 | *r*: 0.983 |
| | 2.5 | 0.592 | CCSI: −0.42 |
| Mixture (17.4:1) | | | |
| 9.2 | | 0.507 | |
| 18.4 | | 0.769 | *Y*-int: $-1.67 \pm 0.016$ |
| 27.6 | | 0.872 | *m*: $1.74 \pm 0.01$ |
| 36.8 | | 0.919 | *r*: 0.999 |
| 46.0 | | 0.944 | CCSI: −0.98 |

Table 3
The restults of median-effect analysis with and without confidence interval for the combination ADP-ribose and ADP using the mutually exclusive equation

| $F_a$ | $CI$ | $\overline{CI}$ | up CIB [a] | low CIB [a] | $\Delta$ | $\delta_\Delta$ | Diagnosis | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | without CIB [a] | with CIB [a] |
| 0.10 | 1.054 | 1.056 | 1.096 | 1.012 | 0.041 | 0.039 | Antag. | Antag. |
| 0.20 | 1.041 | 1.042 | 1.064 | 1.018 | 0.022 | 0.022 | Antag. | Antag. |
| 0.30 | 1.033 | 1.034 | 1.045 | 1.021 | 0.012 | 0.011 | Antag. | Antag. |
| 0.40 | 1.027 | 1.027 | 1.035 | 1.018 | 0.008 | 0.008 | Antag. | Antag. |
| 0.50 | 1.021 | 1.021 | 1.033 | 1.008 | 0.012 | 0.012 | Antag. | Antag. |
| 0.60 | 1.016 | 1.016 | 1.035 | 0.995 | 0.020 | 0.019 | Antag. | Summat. |
| 0.70 | 1.010 | 1.010 | 1.038 | 0.981 | 0.028 | 0.028 | Antag. | Summat. |
| 0.80 | 1.003 | 1.003 | 1.042 | 0.964 | 0.038 | 0.038 | Antag. | Summat. |
| 0.90 | 0.994 | 0.992 | 1.047 | 0.941 | 0.053 | 0.053 | Synerg. | Summat. |
| 0.98 | 0.978 | 0.974 | 1.051 | 0.938 | 0.063 | 0.059 | Synerg. | Summat. |

[a] CIB = Confidence interval boundary.

$CI$ and $\overline{CI}$ coincide with each other with the accuracy ($\Sigma$) in the order of $10^{-2}$ to $10^{-3}$. The magnitude of $\Delta$ and $\delta_\Delta$ for data shown in Table 4 is higher ($\delta_\Delta = 0.42$) than for the data in Table 3 ($\delta_\Delta = 0.06$). Comparing the linear correlation coefficients for each drug alone and their mixture for the initial data shown in Table 1 ($r_1 = 0.998$, $r_2 = 0.999$, $r_3 = 0.999$) and in Table 2 ($r_1 = 0.998$, $r_2 = 0.983$, $r_3 = 0.999$), we note that the lower relative error $\delta_\Delta$ (Table 3) corresponds to the higher linear correlation coefficients. Thus, the linear correlation coefficients influence the magnitude of the confidence interval. However, if we compare the variations of the relative errors of the median-effect parameters:

$$\delta_m = \frac{\text{S.E.}_m}{m} \quad \text{and} \quad \delta_{y\text{-int}} = \frac{\text{S.E.}_{y\text{-int}}}{y\text{-int}}. \tag{7}$$

Table 4
The results of median-effect analysis with and without confidence interval for the combination *o*-phenanthroline and ADP at 17.4 : 1 ratio, using the mutually non-exclusive equation

| $F_a$ | $CI$ | $\overline{CI}$ | up CIB [a] | low CIB [a] | $\Delta$ | $\delta_\Delta$ | Diagnosis | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | without CIB [a] | with CIB [a] |
| 0.10 | 1.096 | 1.123 | 1.411 | 0.780 | 0.315 | 0.287 | Antag. | Summat. |
| 0.20 | 0.874 | 0.884 | 1.010 | 0.740 | 0.134 | 0.153 | Synerg. | Summat. |
| 0.30 | 0.755 | 0.758 | 0.819 | 0.690 | 0.064 | 0.085 | Synerg. | Synerg. |
| 0.40 | 0.670 | 0.670 | 0.713 | 0.627 | 0.043 | 0.064 | Synerg. | Synerg. |
| 0.50 | 0.601 | 0.600 | 0.654 | 0.549 | 0.052 | 0.087 | Synerg. | Synerg. |
| 0.60 | 0.541 | 0.539 | 0.609 | 0.472 | 0.068 | 0.127 | Synerg. | Synerg. |
| 0.70 | 0.482 | 0.480 | 0.565 | 0.397 | 0.084 | 0.175 | Synerg. | Synerg. |
| 0.80 | 0.419 | 0.418 | 0.517 | 0.321 | 0.097 | 0.233 | Synerg. | Synerg. |
| 0.90 | 0.341 | 0.340 | 0.448 | 0.233 | 0.108 | 0.317 | Synerg. | Synerg. |
| 0.96 | 0.224 | 0.222 | 0.388 | 0.155 | 0.111 | 0.418 | Synerg. | Synerg. |

[a] CIB = Confidence interval boundary.

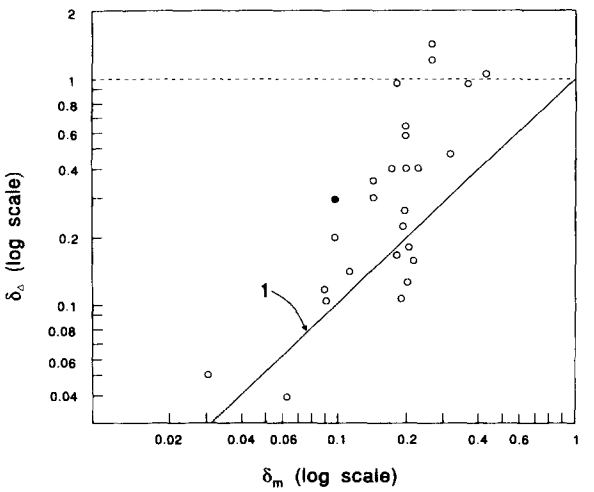


Fig. 1. Dependence of the relative error for the combination index on the relative error for the slope. Open circles are the results of calculations with the real experimental data under the mutually exclusive assumption; dark circles are the results of calculation under the mutually nonexclusive assumption; 1 is the line of the equal values of $\delta_\Delta = \delta_m$.

we find that they vary by a wider range, i.e., $\delta_m = 0.03$ and $\delta_{y\text{-int}} = 0.03$ for the data in Table 1 and $\delta_m = 0.11$ and $\delta_{y\text{-int}} = 0.13$ for the data in Table 2.

The calculations performed with the data for different published combination experiments (Chou and Talalay, 1984; Eriksson and Schinazi, 1986; Schinazi et al., 1986, 1990) as well as unpublished results obtained in our laboratory show that the overall maximum error for each drug alone and in combination, for the slope, $\delta_m$ has the most significance for the *CI* error $\delta_\Delta$. In order to prove this, all the data of 27 different experiments (26 under mutually exclusive and 1 under mutually nonexclusive assumptions) have been presented on the plot: $\log_{10}\ \delta_\Delta$ vs. $\log_{10}\ \delta_m$ (Fig. 1).

In this plot, the *x*-axis corresponds to the overall maximum error for each drug alone and its mixture, and the *y*-axis corresponds to the maximum error for the *CI*, $\delta_\Delta$, for all $F_a$ values. Each point in the figure represents the data from one combination experiment. Line 1 corresponds to the values when $\delta_\Delta = \delta_m$. From Fig. 1 it is evident that for the small slope error, $\delta_m$, the *CI* error is proportional to $\delta_m$. At the same time, when the slope error, $\delta_m$, increases the *CI* error exceeds $\delta_m$ and might be equal to or larger than 1. Thus, a large slope error, $d_m$, might lead to large *CI* errors. Note that in the Chou and Talalay method without confidence interval, one can calculate *CI* values for arbitrarily large errors of the slope.

It should also be noted that the data presented on Fig. 1 were obtained for both mutually exclusive and nonexclusive cases. In the first case ($\alpha = 0$), all the theoretical approaches which are available now provide the same basic equation for the *CI*, so the results obtained are absolute. In the nonexclusive case ($\alpha = 1$), as has been noted, there is a controversial situation about the justification of the Eqn. 1. Nevertheless, as the simulation technique is universal, it can be equally applied to the Chou and Talalay

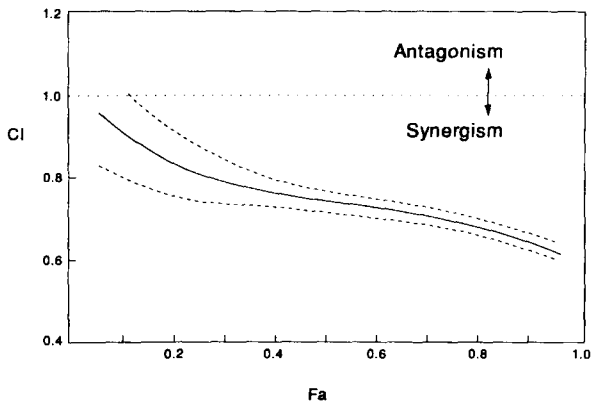


Fig. 2. $F_a$-$CI$ plot with confidence interval for the combination of AZT and PFA at 1 : 1000 ratio against HIV-1 replication in acutely infected human peripheral blood mononuclear cells under a mutually exclusive assumption.

formula, to alternative formulae proposed by Syracuse and Greco (1986), and Lam et al. (1991), or to formulae which may be developed in the future. The unique basic equation for the $CI$ in the nonexclusive case ($\alpha = 1$) can be selected when consensus among these investigators has been achieved.

In order to demonstrate the applicability of the developed approach to the antiviral research one more example is presented in Fig. 2. The initial dose effect data points for this were taken from work performed by our group with 3′-azido-3′-deoxythymidine (AZT) and phosphonoformate (PFA) in human peripheral blood mononuclear (PBM) cells acutely infected with human immunodeficiency virus type 1 (Eriksson and Schinazi, 1989). The median effect parameters which have the greatest effect on $CI$ error were as follows: $\delta_{m1} = 0.09$; $\delta_{m2} = 0.095$; $\delta_{m3} = 0.082$. This case, according to Fig. 1, corresponds to the small slope error $\delta_m \ll 1$, so the maximum $CI$ error in Fig. 2 is fairly small ($\delta_\Delta = 0.11$). Thus, multiple drug effect analysis with confidence interval can be applied successfully in antiviral research.

## 3. Conclusions

In this paper the statistical approach for the calculation of the confidence interval for the $CI$ based on the Monte Carlo Technique was developed. The new parameters which improve the analysis, the modified interaction diagnosis with confidence interval and statistical criteria for the comparison of the results of different agents in combination studies were introduced for the first time. Examples of applications which demonstrate the advantages of developed techniques were presented. The dependence of the confidence interval and relative error for the $CI$ from the variation in the median effect parameters were studied. It was shown that the maximum relative error for the slope has the greatest effect on the confidence interval values. The developed simulation technique is universal, and it might be applied both to the Chou and Talalay formula for the

combination index, to alternative formulations which are available now, or to formulae which might be developed in the future. Nevertheless, further development of the simulation technique is needed. In particular, it is necessary to study the sensitivity of the confidence interval for the *CI* to the assumptions for the probability distribution of the median-effect parameter errors other than Gaussian and uniform [1].

## Acknowledgments

We thank Dr. T.-C. Chou (Sloan-Kettering, New York) for suggesting the use of the Monte Carlo technique to improve the multiple drug effect analysis method, and Dr. D. Goldsman (Georgia Tech, Atlanta) for fruitful discussions. This work was supported in part by the Department of Veterans Affairs, and by the Georgia VA Research Center for AIDS and HIV Infections.

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[1] The Combostat® program has been developed in our laboratory for use on IBM compatible computers to calculate *CI* values with confidence intervals. For further information write to Combostat, 4760 Trevino Circle, Duluth, GA 30136, USA.

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