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Implications and problems in analysing cytotoxic activity of hydroxyurea in combination with a potential inhibitor of ribonucleotide reductase*

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Summary. The cytotoxicity of hydroxyurea in combination with 2,2′-bipyridyl-6-carbothioamide (a potential inhibitor of ribonucleotide reductase) on P388 murine leukemia is reported. Synergistic activity was studied using various interpretations of the isobologram method and the combination index method. We evaluated the pros and cons of these methods and their overall usefulness. In our opinion, to obtain all possible information from a compound association, it is important to choose a formally correct method that (a) can quantitatively evaluate synergism or antagonism, (b) may offer the possibility of averaging final results, (c) needs a minimal amount of experimental data, and (d) is rapid. Moreover, we emphasize both the utility of testing at least three molar ratios of compound association and the importance of carefully choosing the fractional inhibition used in calculating the combination effect. Such evaluation of drug combinations gives information essential to the preparation of new anticancer drug regimens and to the early assessment of biochemical interactions.

Introduction

Over the past few years combination chemotherapy has assumed a crucial role in antineoplastic therapy. Moreover, new methods based on rational drug association should enable further progress. To investigate drug association effects, in vivo models have been extensively used. Today they remain essential for evaluating the comprehensive effects, deriving from several interaction levels, of combination therapy (optimal therapy: Wampler et al. [30]; therapeutic synergy: Venditti and Goldin [29]).

In vitro studies enable a more careful evaluation of the effect (synergism, additivism, antagonism) of drug association on the tumor cell population. Since in vitro studies are also less expensive and faster, they seem to represent the obvious first step in evaluating drug associations [11]. However, a crucial matter in the study of drug combination in vitro involves the way in which the results of association should be analysed. In fact, there is the risk [3, 14] of using methods that result in either inaccurate or false conclusions; nevertheless, a failure to analyse results at all means the loss of precious information.

We studied in vitro the association between hydroxyurea (HU) and 2,2′-bipyridyl-6-carbothioamide (BPYTA). Results were analysed using the analysis methods that presently seem to be more correct and suitable for most systems [4, 9, 27]. In this work we evaluated the advantages in analysing drug-combination results and compared the methods of analysis.

It is generally accepted that the primary mechanism of HU involves the inhibition of the ribonucleotide reductase (RR) M_2 subunit [18]. HU is not highly potent but it has been successfully used in some antileukemic treatments [15, 17]. BPYTA is a new type of conjugate *N**-*N**-*S** tridentate ligand [1], similar in structure to 1-formylisoquinoline thiosemicarbazone, a most active inhibitor of the RR M_2 subunit (see Fig. 1) [18]. Association of these drugs had two goals: (a) to verify the possibility of enhancing HU cytotoxicity by the addition of BPYTA and (b) to study the role of BPYTA's chelating activity in drug interactions.

Materials and methods

Drugs and chemicals. BPYTA was synthesized by P. Franchetti (Department of Chemical Sciences, Camerino University). HU was obtained from the commercial preparation Oncocarbide (Simes). RPMI-1640, newborn calf serum and additive solutions were purchased from Biochrom-Seromed (Berlin, FRG). [^{125}I]-5-iodo-2′-deoxyuridine ([^{125}I]-UdR, 35.4 Ci/mmol) was obtained from Amersham (England).

Drug solubilization. HU was dissolved in bidistilled water and stored at –20°C. BPYTA was solubilized in dimethyl sulfoxide (DMSO) immedi-

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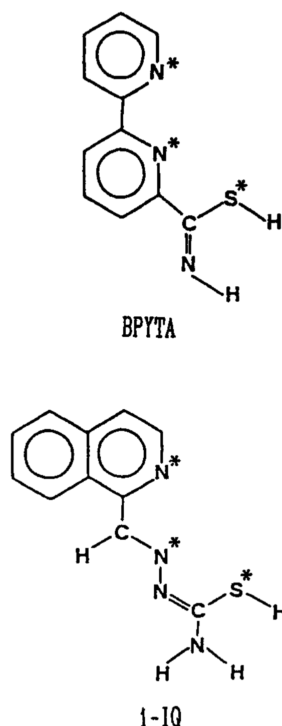

Fig. 1. Structures of 2,2′-bipyridyl-6-carbothioamide (BPYTA), characterized by tridentate ligand system N^*-N^*-S^* and 1-formylisoquinoline thiosemicarbazone (1-IQ)

ately before testing. The highest DMSO concentration used (0.08%) did not have any cytotoxic effect in our testing system.

Cell culture. The P388 murine leukemia cell line was maintained in continuous suspension culture in RPMI-1640 medium (pH 7.2) containing antibiotics (100 IU/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamicin) and supplemented with 10% newborn calf serum, 3 m*M* glutamine, 10 m*M* HEPES buffer and 0.01 µ*M* 2-mercaptoethanol. Cells were grown in a humidified atmosphere of 5% CO_2 and 95% air at 37° C.

In vitro assay. The assay developed for predictive evaluation of tumor chemosensitivity [21] has previously been used in in vitro studies on the antitumor activity of BPYTA and its metal complexes [10]. Briefly, various concentrations of each drug and each combination were placed, in quadruplicate, in flat-bottomed microculture wells with tumor-cell suspension (10^5 cells/well in 0.2 ml) in the same tissue-culture medium and were then incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37° C. After 48 h, DNA synthesis was evaluated by the addition of 0.1 µCi [^{125}I]-UdR per well, along with 2′-deoxy-5-fluorouridine (0.01 µg/well), to the cultured cells for an additional 18 h. This incubation time, at which cells were still growing exponentially, was adopted in line with previous experiments carried out in our laboratory [21] and elsewhere [2]. Harvesting was carried out by a multiple suction-filtration apparatus (Mash II) on a fiberglass filter (Whittaker Co., USA). The filter paper was washed six times with 0.85% NaCl solution and the paper disks containing the aspirate cells were read in a gamma-scintillation counter. Results are expressed as fractions of inhibited radioisotope incorporation in the treated cultures as compared with the untreated controls.

Data analysis. When required, the dose-effect relationship was analysed by the median-effect equation derived by Chou [6, 7]:

$$f_i/(1-f_i) = (D/ID_{50})^m, \quad (1)$$

where D represents the dose (or concentration) of drug that produces a determined f_i on the system, ID_{50} represents the median-effect dose (or concentration) that is required for 50% cytotoxicity, f_i represents the fractional inhibition value and m, the Hill-type coefficient signifying the degree of sigmoid shape of the dose-effect curve. The logarithmic form of Eq. 1 gives the bases for the median-effect plot [7, 8] $x = \log D$ vs $y = \log f_i/(1-f_i)$, which yields the slope (m) and the x-intercept ($\log ID_{50}$). The ID_{50} can be calculated from the antilogarithm of −y intercept/m. If the correlation coefficient for the regression line (R) is >0.9, the equation can be considered to fit the dose-effect relationship. Combination of drug 1 with drug 2 at a determined molar ratio (P:Q) was analysed using Eq. 1, where D ($=D_{1,2}$) represented the sum of the concentration of both drugs and ID_{50} and m were parameters of the association. For a determined f_i produced by the concentration $D_{1,2}$ of the association, the concentration of drug 1 in combination (D_{1c}) was calculated by

$$D_{1c} = (D_{1,2})(P)/(P+Q) \quad (2)$$

and the concentration of drug 2 in combination (D_{2c}) was calculated by

$$D_{2c} = (D_{1,2})(Q)/(P+Q). \quad (3)$$

The synergism, summation or antagonism of drug effects were quantitatively analysed by the multiple-drug-effect analysis developed by Chou and Talalay [9]. The effect of interaction of drug 1 and drug 2 was quantitatively determined by the combination index (CI), which is defined by

$$CI = \frac{(D_{1c})}{(D_1)} + \frac{(D_{2c})}{(D_2)} + \alpha \frac{(D_{1c})(D_{2c})}{(D_1)(D_2)}, \quad (4)$$

where D_1, the dose of drug 1 (or D_2, the dose of drug 2), was calculated by Eq. 1; D_{1c}, by Eq. 2; and D_{2c}, by Eq. 3 for the same f_i. When $m_1 = m_2$ and is equal to $m_{1,2}$, a mutually exclusive drug effect is indicated and $\alpha = 0$; when $m_1 = m_2$ but is $< m_{1,2}$, a mutually nonexclusive drug effect is indicated and $\alpha = 1$; when $m_1 \neq m_2$, exclusiveness of the drug effects cannot be clearly determined, such that the calculation of CI requires the use of both $\alpha = 0$ and $\alpha = 1$. This analysis generates the following combination effect: when CI = 1, summation is indicated; when CI <1, synergism is indicated; and when CI >1, antagonism is indicated. A computer program based on the above equations has been developed in BASIC language on an IBM PC, and it was used in this study for automated analysis of dose-effect data. Each datum shown here is the mean of at least three experiments, analysed separately.

Analysis of drug interactions was also carried out using the isobologram method according to the general line exposed and demonstrated by Berenbaum [4], following Loewe's work [19, 20]. Concentrations of drug 1 at which fractional inhibitions (f_is) were obtained on the cell systems are shown on the x-axis; concentrations of drug 2 at which f_is were obtained on the cell system are shown on the y-axis. A straight line joins points on the respective axes, representing concentrations at which a given f_is or a range of f_i was obtained (calculated data or experimental data). For the same f_i or range of f_is, experimental data points (or calculated data points) of the combination are plotted. If points fall in the area to the right of the straight line, antagonism is indicated; if they also fall on the straight line, additivism is indicated; if one or more points fall in the area to the left of the straight line, synergism is indicated. In other words, if the line joining the lower points representing the chosen f_i is concave upwards, synergism is indicated; if it is concave downwards, antagonism is indicated; if it lies exactly on the straight line, additivism is indicated.

Finally, analysis of drug interaction was performed using the new approach to the isobologram method according to Steel and Peckham [26, 27]. Data were plotted as indicated above (Berenbaum method), but the interpretation of results differed. An envelope of additivity was individualized by plotting three lines (mode I, mode IIa, mode IIb), which were constructed on the basis of the dose-response curves for drugs 1 and 2 at a given f_i [27]. For the same range of f_is, experimental data points of the combination are considered. If points fall only in the area to the right of the envelope, a sub-additive effect (or antagonism) is indicated. If points also fall within the envelope, agents could be acting by the same mechanisms or by independent mechanisms (depending on their nearness to the mode II or mode I line, respectively). Even if only one point falls in the area to the left of the envelope, a supra-additive effect (or synergism) is indicated, i. e. the response is greater than would be expected from any analysis of the individual dose-response curves.

Results

To evaluate drug synergism, as a first step we carried out in vitro tests assessable by isobologram methods according to Berenbaum and Steel and Peckham. Simultaneously, scalar concentrations of HU and BPYTA and 40 concentrations of HU plus BPYTA at different molar ratios were tested. In Figs. 2 and 3 the results obtained from one representative experiment are shown according to Berenbaum and to Steel and Peckham, respectively. Four similar tests substantially confirmed the above results. It was not possible to average data (asymmetric and not quantitatively equal) because means plotted on an isobologram distort the result in comparison with the results of each of the five experiments. Moreover, in the isobologram method according to Steel and Peckham, the envelope of additivity presented a reasonable amount of variation from experiment to experiment.

According to the Berenbaum method, there was a strong synergism between drugs; five black squares in Fig. 2 fell below the straight line joining the ones on the x- and y-axes. However, the meaning of the high inhibition value obtained with 45 μ*M* HU plus 2.7 μ*M* BPYTA on line 2 and the asymmetry of the inhibition value on line 3 is not clear, as points on the same line generally have a similar inhibition value.

Such synergism was also confirmed by elaboration of the results according to Steel and Peckham (Fig. 3a): four black squares fell in the area of supra-additivity. Considering the caution of the method in claiming a synergic effect, such result becomes quite interesting.

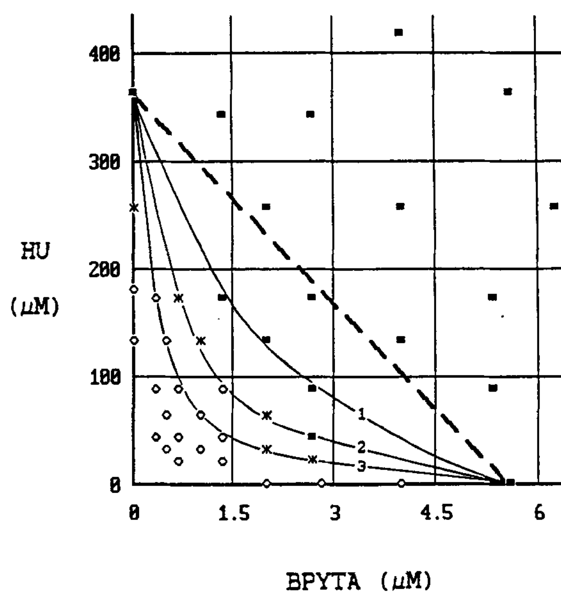

Fig. 2. Synergistic effect of the HU-BPYTA association, shown by the isobologram method according to Berenbaum. ■, concentration giving a high inhibition value ($f_i \geq 0.9$); *, concentration giving an intermediate inhibition value ($0.4 < f_i < 0.9$); ◇, concentration giving a low inhibition value or no inhibition ($f_i \leq 0.4$)

To obtain more exact information, we carried out a series of experiments testing HU-BPYTA at given molar ratios. For each association, scalar concentrations were tested and the dose-effect relationship was analysed using the median-effect equation derived by Chou [6, 7] (see

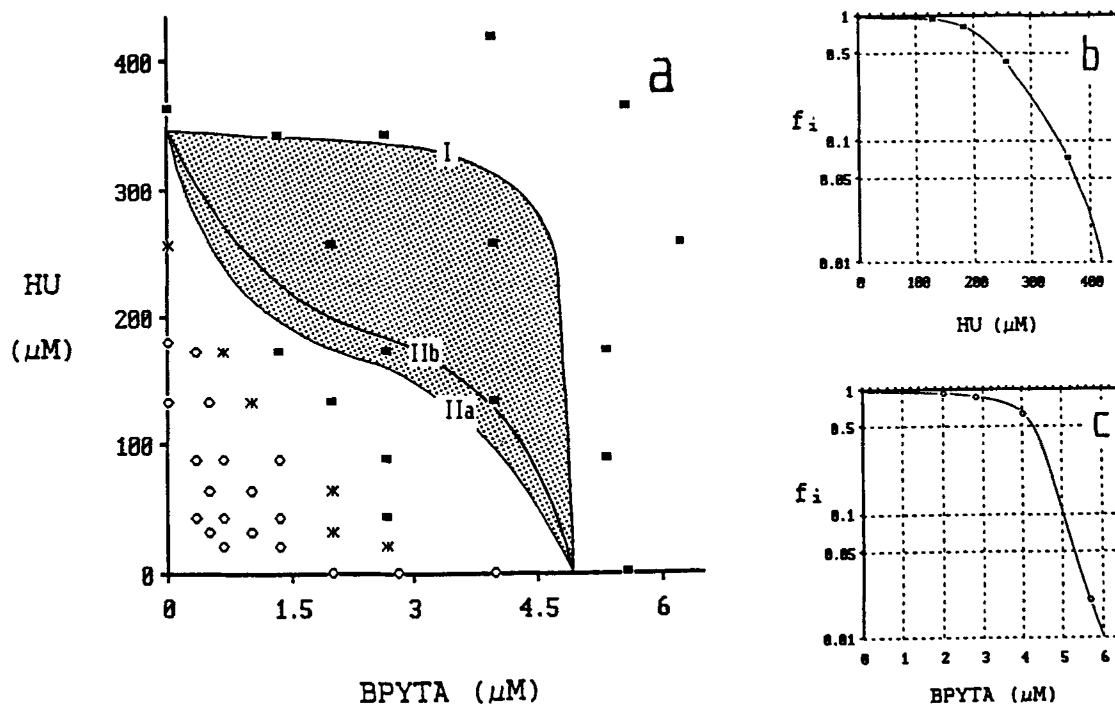

Fig. 3a–c. Synergistic effect of the HU-BPYTA association, shown by the isobologram method according to Steel and Peckham. One significative experiment is shown. The envelope of additivity (*dotted area*) is plotted for $f_i = 0.9$. **a** ■, concentration giving a high inhibition value ($f_i \geq 0.9$); ∗, concentration giving an intermediate inhibition value ($0.4 < f_i < 0.9$); O, concentration giving a low inhibition value or no inhibition ($f_i \leq 0.4$). **b, c** Dose-effect curves of HU and BPYTA, respectively, used in plotting the envelope of additivity for **a** [27]

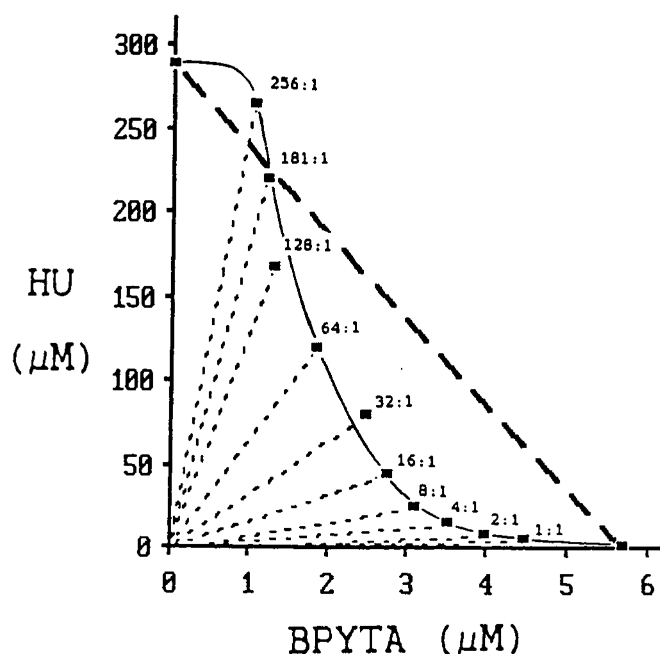

Fig. 4. Synergistic effect of the HU-BPYTA association, shown using the isobologram method (modified). Concentrations giving a fractional inhibition value equal to 0.9 are calculated for compounds and their associations using the median-effect equation derived by Chou; they represent the mean of three values

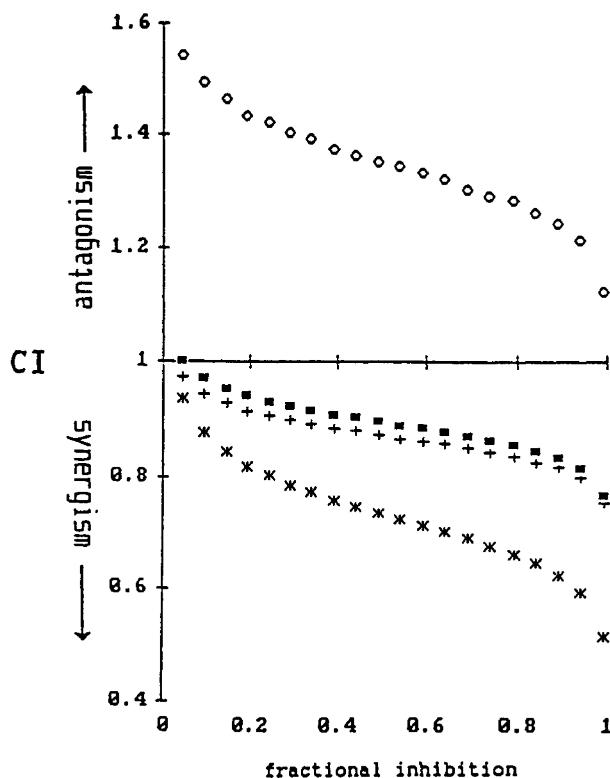

Fig. 5. Effects of the HU-BPYTA association evaluated using the combination index (CI) for 4 significant molar ratios: +, 1:1; ∗, 8:1; ■, 64:1; ○, 256:1. Results of one representative experiment are shown

Materials and methods). Using Eqs. 2 and 3, we calculated $D_{(HU)c}$ and $D_{(BPYTA)c}$ for $f_i = 0.9$. Results are shown in the isobologram in Fig. 4. Each association at a defined molar ratio was tested three times, and only mean values are shown. The ID_{90} for HU and BPYTA represent the weighted mean of values obtained throughout the entire study. The straight line joining these points is considered to be the parameter for additive effect, whereas, according to Steel and Peckham [27], the possibility of using linearization for the dose-effect relationship (R>0.95, always) renders the envelope of additivity unnecessary. Synergism was confirmed, although there was a bimodal curve, with low antagonism at an HU-BPYTA molar ratio of 256:1 and the highest synergism at a molar ratio of 8:1.

The same data used to plot Fig. 4 were used to calculate CI according to Chou and Talalay [9] (Figs. 5, 6). Since the mean $m_{HU} = 7.3 \pm 2.3$ and $m_{BPYTA} = 7.0 \pm 1.4$ and m of each HU-BPYTA association was >10 (Fig. 7), it was assumed that HU and BPYTA are mutually nonexclusive drugs ($\alpha = 1$). CIs calculated for all f_i values obtained at some determined molar ratios are shown in Fig. 5, whereas the CIs calculated for some given f_i values are shown for all molar ratios tested in Fig. 6; results similar to those above were obtained when CIs for $f_i = 0.9$ are considered (dotted columns).

Discussion

Methods

It could be said that all graphs used to illustrate the phenomenon reach the same conclusion. However, the Berenbaum and Steel and Peckham methods (Figs. 2, 3) require numerous experimental data that are difficult to reproduce quantitatively and cannot give information as precise as that obtained by the other methods. This may be the reason why the isobologram method [20] has been and continues to be used by very few authors, despite its correct approach, the authority of the authors who suggested and used it, and its "age".

Indeed, to make the isobologram method practical and to avoid constructing the envelope of additivity, we tried to linearize the dose-effect curves of the compounds tested alone and in association. The processing of data shown in Fig. 4 was notably more precise and required the same number of experimental data used for processing by the first methods. However, a sufficiently precise analysis can be obtained even if the necessary experimental data are reduced. The molar ratios of association could be only three: that between drug concentrations with similar activity (as with HU-BPYTA 64:1) and those with concentrations greatly differing in favour of one or the other drug (as with HU-BPYTA 16:1 and 256:1). With three ratios distributed in this manner, it can be ascertained whether the isobologram curve is unimodal or multimodal. The importance of this evaluation is obvious due to the possible

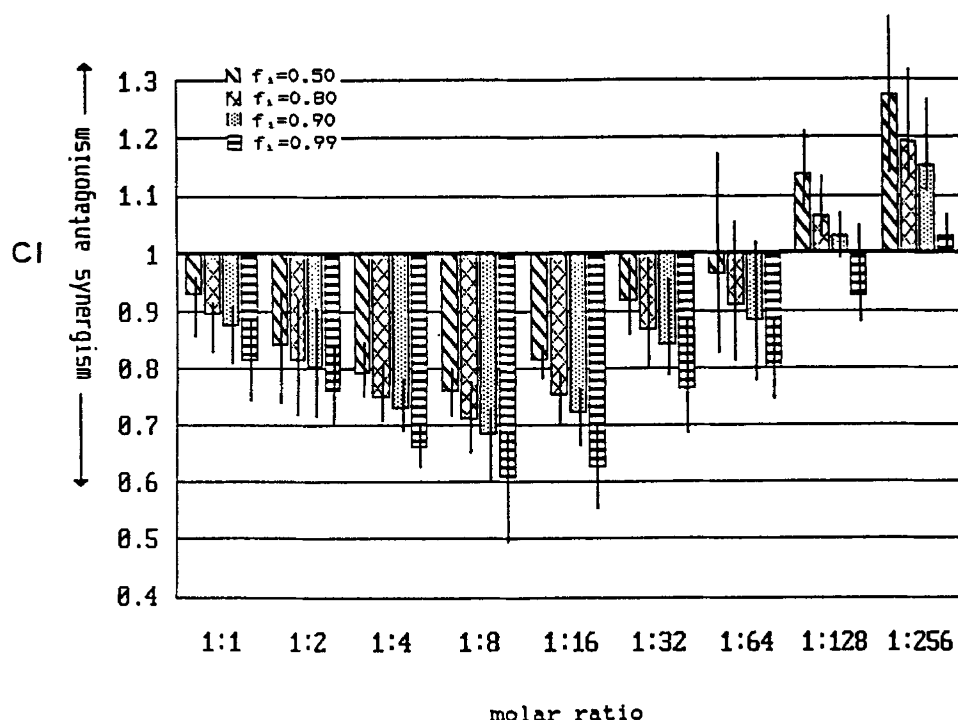

Fig. 6. Quantitative evaluation of effects of the HU-BPYTA association, shown using the combination index (CI) at different molar ratios. CI values corresponding to 4 representative fractional inhibitions are reported. Columns represent the means of results from three experiments; bars represent the highest and lowest values

consequences deriving from both the clinical application of the information on synergism and the development of hypotheses on activity-structure relationships (see discussion of compounds). Thus, it appears that the isobologram method is easy to use with linearization; our data fitted well with the linearization proposed by Chou, but other linearization ways could achieve the same effect.

The data were also analysed with the Chou and Talalay [8, 9] method using the combination index (Figs. 5, 6). Although the theoretical considerations and mathematical processes used by these authors are different from those used in working out the isobologram method [19, 20], the fact that the final results were identical for mutually exclusive drugs is noteworthy. This confirms both the isobologram and the combination index methods. In evaluating mutually nonexclusive drugs, we observed slight quantitative differences. However, in our opinion, the conceptual difference between mutually exclusive and mutually nonexclusive drugs is not clear and needs further discussion.

The principal advantage of the Chou and Talalay method is the fact that the variability of response by the biological system to the individual drugs no longer represents a problem. Indeed, the datum concerning synergic activity (CI) is obtained from one association experiment. The CI value obtained is compared with the value obtained from similar experiments; thus, the final value is the mean CI value obtained in each experiment. The computerization of the method is another advantage of this system. However, it could appear too sophisticated and difficult to understand because only the final results are shown but the experimental data do not appear at all.

The way adopted by Chou and Talalay to the results obtained (Fig. 5) also emphasizes the importance of the f_i value chosen. The CI values obtained by associating HU and BPYTA, calculated for different f_is, are shown in Fig. 6. The isobologram methods also presented similar value fluctuations at different f_i values (data not shown), showing that the choice of f_i is a common, crucial matter. The antagonism shown at high HU-BPYTA ratios is marked at $f_i = 0.5$ and disappears at $f_i = 0.99$. However, at each f_i the association ratio with the highest synergism is 8 : 1. This proves that the question as to which f_i should be chosen is not a problem of minor importance in the quantitative evaluation of low synergism or antagonism.

Thus, in our opinion, the graphic plotting chosen by Chou and Talalay and used by Chang et al. [5] and Durand and Goldie [12] is quite confusing, as Tsai et al. [28] have also claimed. We think it would be better to replace it with a histogram, choosing either the most representative f_is (Fig. 6) or the most suitable ones. It is quite difficult to say which f_i is better; this may also depend on the method used in evaluating cytotoxic activity. It could well lie between 0.8 and 0.99, because these are the only interesting activity values in combination chemotherapy. The use of the histogram is also supported by the need to average at least three similar experiments and by the opportunity of testing at least three molar ratios (see above). An interesting question could involve whether a rise or fall in a CI curve for a compound association might have different meanings or wheter only quantitative values are important. Overall, we believe it incorrect to compare CIs (for different molar ratios or compound associations) calculated using different f_is as Kano et al. [16] have done.

For an overall evaluation of the methods used, their correctness needs to be carefully examined. However, our specific field suggests that we leave this complex problem to others. Therefore we restricted our contribution to a few considerations. Several authors [3, 9, 14] agree that the

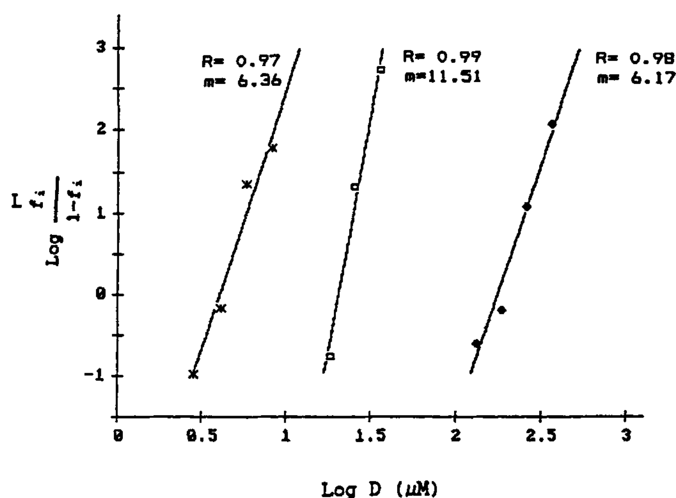

Fig. 7. Cytotoxic activity of HU (●), BPYTA (*) and their association at molar ratio 8 : 1 (□) in a representative experiment on P388 leukemia cells. Results are plotted according to the median-effect equation derived by Chou [6, 7], where f_i is the fractional inhibition of radioisotope incorporation in the treated cultures as compared with untreated controls and D is the concentration of compounds (the sum of concentrations of both compounds for association). Correlation coefficient for the regression line (R) and the Hill-type coefficient (m) are also shown

fractional product method [23] (and other less used methods) can be used only under well-selected conditions, rarely found. The indiscriminate use of this method usually leads to an overestimation of the synergic effect (or to a claim of synergism instead of antagonism), particularly for compounds with a highly sigmoid dose-response curve (as in our compounds). However, Greco [14] recently found Chou and Talalay methods to be sub-optimal, and only future discussion will furnish the elements necessary for evaluating the formal correctness of this new method. Berenbaum [3] demonstrated the inaccuracy of the Steel and Peckham method, and Steel and Peckham [27] did the same for Berenbaum. Overall uniformity of the results obtained could indicate that the methods considered are similar in evaluating drug combinations. Nevertheless, this overlapping could be casual, and it certainly would not have occured if we had studied compounds with antagonistic interactions (the isobologram calculated according to Steel and Peckham differs greatly in this type of evaluation).

Finally, we think it is very important that this issue be studied more closely. Our intention is to repeat this kind of evaluation for another series of compounds and to perform some simultaneous measurements of antitumor and host toxicity effects (see Fodstad and Pihl [13]). It could be possible to evaluate cell-survival curves for leukemic cells, resting bone marrow cells and proliferating marrow cells separately for each agent as well as for various combinations. The evaluation of in vitro data could be done using each method, and the results could then be compared with in vivo results so as to assess which method shows the best fit. This could be a way of empirically selecting the method to be used. It could even be possible to verify which system can better interpret biochemical interactions, thus obtaining another evaluation of the methods. Study in this direction is necessary before the best method can be chosen. However, our work has ascertained that the experimental data must at least include the data necessary to construct the dose-effect curves of the two compounds as well as three of their associations (with molar ratios chosen suitably) and that the experiments should be repeated at least three times.

Drug association

The results obtained show that synergism between HU and BPYTA is good at molar ratios between 16 : 1 and 4 : 1. In other words, it appears that relatively high BPYTA concentrations are active when associated with completely inactive HU concentrations (i. e. low doses of HU enhance BPYTA's activity but not vice versa). However, when observing the phenomenon from the molar ratio point of view alone, we notice that the closer the ratio is to the unit, the more the positive interaction is inclined to increase.

It is well known that the association of compounds with inhibition activity on RR does not necessarily involve synergism or antagonism [22]. We also know that the activity of HU is enhanced by its association with chelators [23–25]. After analysing the curve of HU and BPYTA interaction, we could conclude that BPYTA is synergic with HU due to the chelating activity. However, antagonism occurs when the chelating activity of BPYTA becomes insignificant (high HU-BPYTA molar ratio) and the probable activity on RR is therefore predominant. Studies are being carried out on RR to test the exactness of the proposed hypothesis.

References

1. Antonini I, Claudi F, Cristalli G, Franchetti P, Grifantini M, Martelli S (1981) *N*-N*-S** tridentate ligand system as potential antitumor agents. J Med Chem 24: 1181
2. Asantila T, Toivanen P (1974) Potentation by fluorodeoxyuride of ^{125}deoxyuridine uptake by human and chicken lymphocytes in the quantitation of mitogenic response. J Immunol Methods 6: 73
3. Berenbaum MC (1981) Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 35: 269
4. Berenbaum MC (1985) The expected effect of a combination of agents: the general solution. J Theor Biol 114: 413
5. Chang TT, Gulati S, Chou TC, Colvin M, Clarkson B (1987) Comparative cytotoxicity of various drug combinations for human leukemic cells and normal hematopoietic precursors. Cancer Res 47: 119
6. Chou TC (1976) Derivation and properties of Michaelis-Menten type and Hill type equations for reference ligands. J Theor Biol 39: 253
7. Chou TC (1977) On the determination of availability of ligand binding sites in steady-state systems. J Theor Biol 65: 344
8. Chou TC, Talalay P (1981) Generalized equations for the analysis of inhibitions of Michaelis-Menten and higher-order kinetic systems with two or more mutually exclusive and nonexclusive inhibitors. Eur J Biochem 115: 207
9. 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
10. Cristalli G, Franchetti P, Nasini E, Vittori S, Grifantini M, Barzi A, Lepri E, Ripa S (1988) Metal(II) complexes of 2,2′-bipyridyl-6-carbothioamide as anti-tumor and anti-fungal agents, Eur J Med Chem 23: 301

11. Damon LE, Cadman EC (1988) The metabolic basis for combination chemotherapy. Pharmacol Ther 38: 73
12. Durand RE, Goldie JH (1987) Interaction of etoposide and cisplatin in an in vitro tumor model. Cancer Treat Rep 71: 673
13. Fodstad O, Pihl A (1980) Synergistic effect of Adriamycin and ricin on L1210 leukemic cells in mice. Cancer Res 40: 3735
14. Greco WR (1987) The assessment of synergism, antagonism and additivity: a unified optimal approach (abstract). Proc Annu Meet Am Assoc Cancer Res 28: 426
15. Hussey H, Abrahams JP (1975) Combined therapy in advanced head and neck cancer: hydroxyurea and radiotherapy. Progr Clin Cancer 6: 79
16. Kano Y, Ohnuma T, Okano T, Holland JF (1988) Effects of vincristine in combination with methotrexate and other antitumor agents in human acute lymphoblastic leukemia cells in culture. Cancer Res 48: 351
17. Kennedy BJ (1972) Hydroxyurea therapy in chronic myelogenous leukemia. Cancer 29: 1052
18. Lien EG (1987) Ribonucleotide reductase inhibitors as anticancer and antiviral agents. Prog Drug Res 31: 101
19. Loewe S (1928) Die Quantitation-Probleme der Pharmakologie. Ergeb Physiol 27: 47
20. Loewe S (1953) The problem of synergism and antagonism of combined drugs. Arzneimittelforsch 3: 285
21. Menconi E, Lepri E, Bonmassar E, Frati L, Barzi A (1986) An in vitro assay for evaluating chemosensitivity of leukemia cells: preclinical studies. Int J Tiss React 8 (6): 485
22. Sato A, Cory JG (1981) Evaluation of combinations of drugs that inhibit Ehrlich tumor cell ribonucleotide reductase. Cancer Res 41: 1637
23. Sato A, Bacon PE, Cory JG (1984) Studies on the differential mechanisms of inhibition of ribonucleotide reductase by specific inhibitors of the non-heme iron subunit. Adv Enzyme Regul 22: 231
24. Satyamoorthy K, Chitnis M, Basrur V (1986) Sensitization of P388 murine leukemia cells to hydroxyurea cytotoxicity by hydrophobic iron-chelating agents. Anticancer Res 6: 329
25. Satyamoorthy K, Chitnis M, Basrur V, Advani SH (1986) Potentiation of hydroxyurea cytotoxicity in human chronic myeloid leukemia cells by iron-chelating agent. Leukemia Res 10(11): 1327
26. Steel GG (1979) Terminology in the description of drugradiation interactions. Int J Radiat Oncol 5: 1145
27. Steel GG, Peckham MJ (1979) Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat Oncol 5: 85
28. Tsai CM, Gazdar AF, Venzon DJ, Steinberg SM, Dedrick RL, Mulshine JL, Kramer BS (1989) Lack of in vitro synergy between etoposide and *cis*-diamminedichloroplatinum (II). Cancer Res 49: 2390
29. Venditti JM, Goldin A (1964) Drug synergism in antineoplastic chemotherapy. Adv Chemother 1: 397
30. Wampler GL, Carter WH Jr, Williams VR (1978) Combination chemotherapy: arriving at optimal treatment levels by incorporating side effect constraints. Cancer Treat Rep 62(3): 333
31. Webb JL (1963) Enzyme and metabolic inhibitors. Academic Press, New York, vol. 1, pp 66, 488