Cancer Chemother Pharmacol (1993) 31: 259–264

Cancer Chemotherapy and Pharmacology

Quantitation of the synergistic interaction of edatrexate and cisplatin in vitro*

Ting-Chao Chou[1], Queng-Hui Tan[1], and Francis M. Sirotnak[2]

[1] Laboratories of Biochemical Pharmacology and [2] Molecular Therapeutics, Memorial Sloan-Kettering Cancer Center New York, New York 10021, USA

Received 20 April 1992/Accepted 14 August 1992

Summary. Cytotoxicity studies combining edatrexate (EDX) and cisplatin (Cis-Pt) were carried out in HL-60 cells in vitro as a retrospective analysis of the same combination in animal models and as a prospective study of this combination for future clinical trials. For purposes of comparison, parallel studies were carried out using methotrexate (MTX) and Cis-Pt. Dose-effect relationships were analyzed by the median-effect principle and the combination index-isobologram technique. EDX was the most cytotoxic agent of the three examined. The doses effective in 50% inhibition of the cell proliferation (ED_{50} values) for EDX, MTX, and Cis-Pt were 0.001, 0.0043, and 1.08 μM, respectively. Synergism occurred at effect levels corresponding to greater than 65% inhibition of cell growth by EDX + Cis-Pt, with an increase in synergism being observed at high doses. By contrast, MTX + Cis-Pt exhibited moderate synergism, with a decrease in synergism being noted at high doses. Preceding one drug by another for 4 h during the 48-h incubation period did not result in synergism greater than that produced by simultaneous exposure to both drugs for both pairs of combinations. Due to the synergism arising from these combinations, the ED_{90} values can be reduced by as many as 52 and 7.3 times for Cis-Pt and EDX, respectively, as compared with only 4.0 and 1.9 times for Cis-Pt and MTX, respectively. The calculation of these drug interactions was carried out automatically with the use of computer software and was also illustrated by a sample calculation performed without computer simulation.

* This work was supported in part by NCI grant CA18856 and by the Elsa U. Pardee Foundation

Abbreviations: MTX, methotrexate; EDX, edatrexate, 10-ethyl-10-deaza-aminopterin; Cis-Pt, cisplatin, *cis*-diamminedichloroplatinum(II); CI, combination index; DRI, dose-reduction index

Correspondence to: T.-C. Chou

Introduction

10-Ethyl-10-deaza-aminopterin (edatrexate or EDX) is a new folate analog that is more efficacious than methotrexate (MTX) against both murine ascitic and solid tumors and human carcinoma xenografts in vivo [12, 15, 16]. EDX has been evaluated in phase I and II clinical studies [10, 14]. Combination of EDX with cisplatin or alkylating agents in experimental models has produced markedly increased survival in mice bearing L1210 leukemia, E0771 mammary carcinoma, and T241 fibrosarcoma as compared with each drug alone [13]. Combinations with EDX have yielded markedly superior therapeutic activity as compared with combinations including MTX instead of EDX [13].

Earlier studies have shown that EDX is equivalent to MTX as an inhibitor of dihydrofolate reductase but is more effectively transported and polyglutaminated in most tumor cells. This results in a greater accumulation of cytotoxic EDX polyglutamates in tumor cells than in normal proliferative intestinal epithelial cells [16] along with a corresponding decrease in DNA synthesis. By contrast, cisplatin, a potent antitumor agent, causes by direct action inter- or intrastrand cross-linking of DNA [9, 18]. In addition to minimal myelosuppression, mucositis is the most common toxicity produced by EDX [10, 14], whereas nausea, vomiting, and renal toxicity are the dose-limiting side effects of cisplatin treatment [1, 11]. The favorable experimental therapeutic effects noted in vitro and in vivo, the different modes of action, and the nonoverlapping toxicities [1, 14] observed between EDX and cisplatin make them a suitable pair of agents for clinical combination chemotherapy.

In general, several pitfalls are involved in the evaluation of combined drug effects in terms of synergism or antagonism. Obviously, an additive effect (i.e., no synergism or antagonism) is not the simple arithmetic sum of the effects of two drugs. For example, if drug 1 and drug 2 each inhibits growth by 60% (f_a = 0.6), the combined effect (if additive) cannot be 120% inhibition (i.e., exceeding 100% inhibition). Furthermore, calculation of additive effects by

the fractional-product method [17] can also lead to erroneous conclusions. For example, in the above case, $(1 - 0.6)(1 - 0.6) = 0.16$, $1 - 0.16 = 0.86$, the additive effect of 86% inhibition expected on the basis of this calculation does not take into account the shape of the dose-effect curve (e.g., if it is sigmoidal). That one drug plus another drug produces an effect greater than that induced by each drug alone does not necessarily indicate synergism, since each drug plus itself may also produce a greater effect.

Ironically, an unambiguous definition of "additive effect" is the prerequisite for any determination of "synergism" or "antagonism." For in vivo experimental or clinical combination therapy, the action of two drugs in combination that produce a therapeutic effect superior to that of each drug alone is frequently termed "therapeutic synergy" [5]. In these studies, both the therapeutic end result and the toxicity to the host are taken into account simultaneously and contingently. Such evaluation of a combined drug effect in vivo is pragmatic but frequently nonquantitative due to limitations at the whole-animal level when the end points of measurement are subjective or when dose-effect parameters are not available. Although the potency of the drug(s) is taken into account, the shape(s) of the dose-effect curve(s) is frequently ignored. Thus, "therapeutic synergy" is often an issue of selectivity of effects (i.e., efficacy versus toxicity) rather than a matter of synergism since "synergy" is, in fact, not determined [5]. As indicated earlier, "therapeutic synergy" may be a result of real synergy, an additive effect, or even an antagonistic effect when two drugs produce nonoverlapping toxicity [5].

In the present study, we applied the combination index-isobologram method [5–8] to analyze the experimental results obtained in in vitro drug-combination studies on EDX or MTX and cisplatin using human HL-60 promyelocytic leukemic cells to determine synergism or antagonism quantitatively. These investigations were initiated to evaluate the interaction between these agents that might explain the markedly increased therapeutic activity of EDX (but not MTX) with cisplatin that had been observed in animal tumor studies [13]. This method allows quantitative determination of the degrees of synergism occurring at different drug concentrations, at different effect levels, for different regimens, and at different combination molar ratios. Computer software [2, 3] based on the median-effect principle and the combination index-isobologram equations [5–8] were used for automated analysis. A sample calculation was also carried out without the use of computer simulation.

Materials and methods

Cells and cell culture. The human promyelocytic leukemia cell line HL-60 was obtained from Dr. R. C. Gallo of the National Cancer Institute (Bethesda, Md.). The cell line was cultured at an initial density of 4×10^5 cells/ml. The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37° C in RPMI-1640 media (Gibco-BRL, Grand Island, N. Y.) supplemented with penicillin (100 IU/ml)/streptomycin (100 µg/ml; Gibco-BRL) and 10% heat-inactivated fetal bovine serum.

Compounds. EDX was synthesized as described previously [15]. *cis*-Diamminedichloroplatinum(II) (cisplatin, Cis-Pt) and MTX were obtained from Sigma Chemical Company (St. Louis, Mo.).

Cell-growth inhibition. HL-60 cells at 4×10^5 cells/ml were incubated at 37° C with 5–8 sequentially diluted drug concentrations by continuous exposure for 3 days. Cells were counted with a hemocytometer, and viability was determined by trypan blue exclusion at 72 h or at other time points as specified. The relationship between the dose and the number of viable cells for particular compounds or combinations thereof was analyzed using a computer program based on the median-effect equation [5] as described below.

Assessment of synergy. The median-effect principle [4, 5] was used to determine the dose-effect parameters for EDX (or MTX), Cis-Pt, or their mixtures at different combination ratios. Each drug or mixture was 2- to 4-fold serially diluted to generate a dose-effect relationship in the cytotoxicity assays. The median-effect equation is expressed as:

$$(f_a)/(f_u) = (D/D_m)^m \text{ or}$$
$$\log (f_a/f_u) = m \log (D) - m \log (D_m). \quad (1)$$

For EDX, D is the dose of EDX, D_m is the median-effect dose (ED_{50}) of EDX, f_a is the fractional inhibition (e.g., $f_a = 0.9$ for 90% inhibition by EDX), f_u is the fraction unaffected ($1 - f_a$), and m is the coefficient signifying the shape of the dose-effect curve for EDX ($m = 1, >1$, and <1 indicates a hyperbolic, sigmoidal, and negatively sigmoidal curve, respectively). The median-effect plot (based on Eq. 1) of $x = \log(D)$ vs $y = \log [f_a/(1 - f_a)]$ yields a straight line with a slope (m) and an x-intercept of $\log (D_m)$. Thus, the parameters (m and D_m for EDX) can be easily determined. The same procedure was carried out for MTX, Cis-Pt, and their mixtures. The conformity of the experimental data to the median-effect principle of the mass-action law is automatically provided by the computer printout [2] in terms of the linear correlation coefficient (r value) of the median-effect plots.

From the m and D_m values, the isoeffective dose (D_x) for any effect level (e.g., ED_{70} for $f_a = 0.7$, ED_{90} for $f_a = 0.9$ ...), for each drug, or for their combinations can easily be determined by the following rearrangement of Eq. 1:

$$D_x = D_m[f_a/(1 - f_a)]^{1/m}. \quad (2)$$

Using parameters m and D_m and Eq. 2, the computer software [2, 3] automatically calculates D_x values for all designated f_a values (0.01–0.99) and provides graphic representations of dose-effect curves and the median-effect plots.

Synergism or antagonism for EDX (or MTX) plus Cis-Pt is determined on the basis of the multiple drug-effect equation of Chou and Talalay [6–8]. This is quantitated by the combination index (CI), where $CI < 1$, $= 1$, and > 1 indicates synergism, additive effect, and antagonism, respectively. Based on the classic isobologram for mutually exclusive effects relative to the end point of measurement, the CI value for x% inhibition is calculated as:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2}. \quad (3)$$

For example, in this study at 90% inhibition, $(D)_1$ represents EDX; $(D)_2$ represents Cis-Pt; $(D_x)_1$ and $(D_x)_2$ are the doses for 90% inhibition by EDX and Cis-Pt, respectively, which can be obtained from Eq. 2; and $(D)_1$ and $(D)_2$ are the doses in combination that also inhibit cell growth by 90% (isoeffective as compared with the single drugs alone). If the calculation is based on the conservative isobologram equation, assuming that the effects of two drugs are mutually nonexclusive (e.g., totally independent relative to the end point of measurement), then an approximated third term designated as the product of the first two terms should be added to Eq. 3. For simplicity, CI values obtained from the classic isobologram equation (Eq. 3) have frequently been used, but the underlying assumption needs to be stated. Computer software [2, 3] generates f_a-CI tables or f_a-CI plots and isobolograms for any specified effect levels for both mutually exclusive and mutually nonexclusive assumptions.

The dose-reduction index (DRI) defines the extent (folds) of dose reduction are possible in a combination for a given degree of effect as compared with the dose of each drug alone [2, 5]: $(DRI)_1 = (D_x)_1/(D)_1$ and $(DRI)_2 = (D_x)_2/(D)_2$; therefore, the relationship between DRI and CI is expressed as

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D_2)}{(D_x)_2} = \frac{1}{(DRI)_1} + \frac{1}{(DRI)_2}. \quad (4)$$

Table 1. Inhibitory effect of Cis-Pt and EDX and their combinations on HL-60 cell growth during 48 h exposure[a]

Drug and concentration		Fractional inhibition	Parameters[b]	Combination index (CI)[c]
Cis-Pt (μM)	EDX (μM)	(f_a)		
7.5		0.949	D_m = 0.941 μM	
5.0		0.907	m = 1.215	
2.5		0.695	r = 0.980	
1.25		0.432		
0.5		0.288		
0.25		0.195		
0.125		0.102		
	0.1	0.890	D_m = 0.001 μM	
	0.0125	0.873	m = 0.610	
	0.0042	0.839	r = 0.869	
	0.0021	0.661		
	0.00105	0.491		
	0.00053	0.212		
0 h, 0 h[d]:				
0.125	0.00625	0.890	D_m = 0.0439 μM	0.210[g]
0.0666	0.00333	0.814	+ 0.0022 μM	0.293
0.04438	0.00222	0.627	m = 2.368	0.895
0.02962	0.00148	0.186	r = 0.934	15.3
0 h, 4 h[e]:				
0.25	0.0125	0.873	D_m = 0.051 μM	0.534
0.125	0.00625	0.839	+ 0.0026 μM	0.415
0.0625	0.003125	0.729	m = 1.528	0.594
0.0417	0.0021	0.432	r = 0.948	3.04
0.03333	0.00166	0.348		4.34
0.0222	0.00111	0.144		18.9
4 h, 0 h[f]:				
0.25	0.0125	0.898	D_m = 0.0364 μM	0.365
0.0625	0.00625	0.839	+ 0.0018 μM	0.208
0.0417	0.0021	0.636	m = 1.332	0.794
0.0333	0.00166	0.381	r = 0.920	3.42
0.0222	0.00111	0.203		9.58
0.0111	0.00056	0.195		5.25

[a] Incubation conditions are described in Materials and methods

[b] The median-effect plot parameters D_m, m, and r are described in Materials and methods; D_m and m values are used for calculating the CI values

[c] A CI value of <1, 1, and >1 indicates synergism, additivity, and antagonism, respectively. As based on the classic isobologram equation [5–8], CI can be calculated by Eq. 3:
$CI = (D)_1/(D_x)_1 + (D)_2/(D_x)_2$,
where $D_x = D_m[f_a/(1\text{-}f_a)]^{1/m}$

[d] Two drugs in 20 : 1 molar ratio were added simultaneously

[e] Cis-Pt and EDX were added at 0 and 4 h, respectively, for a final molar ratio of 20 : 1

[f] Cis-Pt and EDX were added at 4 and 0 h, respectively, for a final molar ratio of 20 : 1

[g] Sample calculation for the CI values of 0.125 μM Cis-Pt + 0.00625 μM EDX, which inhibit HL-60 cell growth by 88.98% (f_a = 0.8898). On the basis of Eq. 2, for Cis-Pt alone to inhibit cell growth by 88.98% would require $[D_{0.8898}]_{Cis\text{-}Pt} = (D_m)_{Cis\text{-}Pt}\ [0.8898/(1\text{-}0.8898)]^{1/1.215}$ = 0.9411 μM × 5.5795 = 5.2508 μM, and for EDX alone to inhibit cell growth by 88.98% would require $[D_{0.8898}]_{EDX}$ = 0.00109 μM $[0.8898/(1\text{-}0.8898)]^{1/0.6098}$ = 0.0335 μM. Therefore:

$$CI = \frac{0.125\ \mu M}{5.2508\ \mu M} + \frac{0.00625\ \mu M}{0.0335\ \mu M} = 0.210 \text{ at } 88.98\% \text{ inhibition.}$$

Computer software [2, 3] was used on an IBM PC for automated calculation and simulation

Table 2. Inhibitory effect of Cis-Pt and MTX and their combinations on HL-60 cell growth during 48 h exposure[a]

Drug and concentration		Fractional inhibition	Parameters[b]	Combination index(CI)[c]
Cis-Pt (μM)	MTX (μM)	(f_a)		
5.0		0.921	D_m = 1.230 μM	
2.5		0.737	m = 1.659	
1.25		0.487	r = 0.998	
0.52		0.211		
0.25		0.066		
	0.125	0.868	D_m = 0.0043 μM	
	0.0625	0.855	m = 0.685	
	0.39	0.855	r = 0.921	
	0.26	0.829		
	0.0625	0.658		
	0.0031	0.316		
0 h, 0 h[d]:				
1.250	0.0625	0.882	D_m = 0.0324 μM	1.075
0.780	0.039	0.879	+ 0.0016 μM	0.692
0.52	0.026	0.868	m = 0.616	0.518
0.347	0.0174	0.842	r = 0.962	0.452
0.25	0.0125	0.790		0.512
0.125	0.00625	0.645		0.678
0 h, 4 h[e]:				
2.5	0.125	0.934	D_m = 0.0870 μM	1.012
1.250	0.0625	0.829	+ 0.00435 μM	1.834
0.625	0.0313	0.816	m = 0.727	1.032
0.260	0.013	0.697	r = 0.962	1.019
0.1735	0.0087	0.684		0.739
0.125	0.00625	0.513		1.440
4 h, 0 h[f]:				
2.5	0.125	0.895	D_m = 0.0129 μM	1.832
1.25	0.0625	0.868	+ 0.00065 μM	1.247
0.625	0.0313	0.868	m = 0.425	0.623
0.26	0.013	0.790	r = 0.966	0.532
0.1735	0.0087	0.750		0.477
0.125	0.00625	0.697		0.490

[a–f] See footnotes to Table 1, except that EDX has been replaced by MTX

Results

Single-drug efficacy and parameters

Two experiments carried out separately at different times yielded consistent results for the average linear correlation coefficients, the actual values being 0.895 ± 0.026 for the two folate analogs, 0.989 ± 0.009 for Cis-Pt, and 0.949 ± 0.005 for their mixtures. As shown in Tables 1 and 2, EDX was more cytotoxic than MTX or Cis-Pt. The D_m values (IC_{50} values) for EDX, MTX, and Cis-Pt as single agents were 0.001, 0.0043, and 1.08 μM, respectively, whereby both folate analogs resulted in negative sigmoidal curves (m<1) and Cis-Pt produced sigmoidal curves (m>1). The m values indicate that both EDX and MTX gave flat dose-effect relationships whereas Cis-Pt yielded more steep dose-effect relationships. The m and D_m values were used to calculate synergistic/antagonistic interactions.

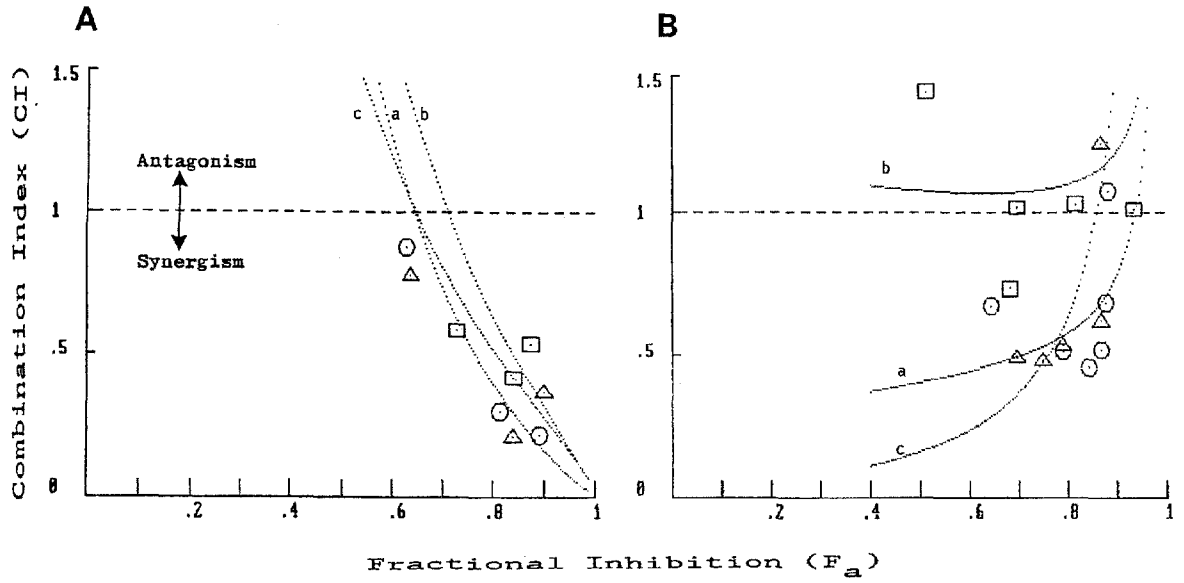

Fig. 1 A, B. F_a-CI plots for **A** EDX + Cis-Pt and **B** MTX + Cis-Pt at a 20:1 combination molar ratio. The symbols shown indicate the actual combination data points obtained when HL-60 cells were simultaneously exposed for 48 h to both EDX (or MTX) and Cis-Pt (○), when Cis-Pt was added at 0 h and EDX (or MTX) was added at 4 h (□), and when EDX (or MTX) was added at 0 h and Cis-Pt was added at 4 h (△). The total incubation period was 48 h. The *dotted curves* derive from a computer simulation carried out for the three regimens using Eqs. 2 and 3. Curves *a*, *b*, and *c* represent the same regimens as do the symbols (○), (□), and (△), respectively. The actual combination data points mainly show the distribution of synergism/antagonism in the f_a-CI plot, and the combination simulation mainly indicates the trend of CI at different f_a values

Drug-combination studies

For the combination of Cis-Pt + EDX, simultaneous exposure and sequential exposure, whereby one drug preceded another by a 4-h interval, yielded similar patterns in f_a-CI plots (Fig. 1A). All showed synergism at effect levels exceeding 65% inhibition, but antagonism was apparent at lower effect levels. For chemotherapeutic purposes, an inhibitory effect of greater than 90% is generally considered to be necessary for the achievement of useful treatment; thus, the antagonism observed at low effect levels may not have practical implications. The degrees of synergism observed between Cis-Pt and EDX were considerably greater than those noted for Cis-Pt and MTX combinations (Fig. 1, Table 3). Computer simulation (Table 3) suggests that simultaneous exposure of cells to EDX + Cis-Pt yielded

Table 3. Computer-simulated CI and DRI values for various combinations at 50%, 75%, 90%, and 95% inhibition of HL-60 cell growth[a]

Drug combination	Regimen of drug addition	DRI values[b] at inhibition of				CI values[c] at inhibition of			
		50%	75%	90%	95%	50%	75%	90%	95%
Cis-Pt	0 h	21.54[b]	33.46	51.86	70.10	2.042[c]	0.554	0.157	0.070
+ EDX	0 h	0.50[b]	1.91	7.27	18.06	(2.135)[c]	(0.509)	(0.159)	(0.070)
Cis-Pt	0 h	18.45	22.21	26.73	30.32	2.384[c]	0.834	0.305	0.161
+ EDX	4 h	0.43	1.27	3.74	7.81	(2.511)	(0.870)	(0.315)	(0.165)
EDX	0 h	25.87	28.00	30.32	32.00	1.701	0.662	0.269	0.153
+ Cis-Pt	4 h	0.60	1.60	4.24	8.25	(0.177)	(0.684)	(0.776)	(0.156)
Cis-Pt	0 h	37.96[b]	12.36	4.03	1.88	0.402[c]	0.530	0.786	1.139
+ MTX	0 h	2.66[b]	2.23	1.86	1.65	(0.412)[c]	(0.566)	(0.919)	(1.463)
Cis-Pt	0 h	14.14	6.05	2.59	1.46	1.079	1.082	1.220	1.469
+ MTX	4 h	0.99	1.09	1.20	1.28	(1.150)	(1.234)	(1.542)	(2.006)
MTX	0 h	95.14	13.89	2.03	0.55	0.160	0.366	1.560	3.902
+ Cis-Pt	4 h	6.67	2.50	0.94	0.48	(0.162)	(0.500)	(2.085)	(7.694)

[a] Incubation was carried out for 48 h under the conditions described in Materials and methods. Dose-effect relationships as shown in Table 1 were analyzed by the median-effect plot [7] using computer software [2, 3] for automated determination of parameters (D_m and m), statistics (*r*), dose-reduction index (DRI), and combination index (CI)

[b] DRI represents the extent (folds) of dose reduction that is possible in a combination for a given degree of effect as compared with the dose of each drug alone (see Eq. 4). The first and second entries are for Cis-Pt and for EDX or MTX, respectively

[c] See footnotes f and g to Table 1 for the sample procedure of calculating CI values. The first entry of CI values are based on the classic isobologram equation (Eq. 4). The CI values shown in parentheses are based on a conservative isobologram equation [5] which has an approximated third term consisting of the product of the first two terms of Eq. 4

slightly greater synergism (i.e., low CI values) than did incubations with EDX followed 4 h later by Cis-Pt, which in turn yielded slightly more synergism than did exposure to Cis-Pt followed 4 h later by incubation with EDX. Similar patterns were observed for MTX + Cis-Pt (Table 3). Thus, the simultaneous presence of both drugs or preexposure to antifolate for 4 h produced greater synergism than did preincubation with Cis-Pt for 4 h followed by the addition of either EDX or MTX.

Although synergism corresponding to CI<1 always yields a favorable dose-reduction index (DRI>1) for one or both drugs, depending on the combination ratio used, slight antagonism may sometimes yield favorable DRI values. For example, on the second-to-last row of Table 3 at 90% inhibition levels, the CI value for MTX + Cis-Pt is 1.220, and yet the DRI value of MTX is 2.59 and that of Cis-Pt is 1.20 [this can be confirmed by CI = (1/2.59) + (1/1.20) = 0.386 + 0.834 = 1.220]. Thus, slight antagonism may nevertheless sometimes warrant the use of a particular drug combination, since the dose of each drug required for a given effect may be reduced to some extent in the combination as compared with each drug alone. This can be especially useful when two drugs such as EDT and Cis-Pt produce mainly nonoverlapping toxicities.

The DRI values for actual combination data points can be calculated and those for selected effect levels can be simulated. The simulated DRI values at ED_{50}, ED_{75}, ED_{90}, and ED_{95} are given in Table 3. Overall, Cis-Pt + EDX produced much higher DRI values and lower CI values than did Cis-Pt + MTX at high effect levels (ED_{75} or greater), regardless of the drug-exposure regimens.

Discussion

Because of their simple, flexible, rapid, and economic features, investigations of drug combinations in vitro can frequently be used for prospective or retrospective studies in animal systems or in clinical settings. Also, in vitro studies may provide the rationale for drug combinations based on quantitation of synergism or antagonism. Due to the multitude of effects of each drug alone, drug interactions are frequently suggestive or speculative. However, the in vitro system can be oversimplified, which may exclude direct extrapolation of the data to animal models or clinical situations; on the other hand, it allows flexible dissection of many facets that are relevant to drug combinations.

In the present studies, by using the median-effect principle and the combination index-isobologram technique, we were capable of analyzing the effect of EDX or MTX used alone or in combination with Cis-Pt and of numerically exploring many aspects of these combinations qualitatively and quantitatively. For instance, we showed that the synergism between EDX and Cis-Pt is much greater than that between MTX and Cis-Pt as determined either by computer simulation or by actual combination data points (Fig. 1) obtained in vitro, which is consistent with findings obtained in animal models in vivo [12, 15]. We also demonstrated that synergism is restricted to specific effect levels and dose levels. In these studies, we determined as well how much dose reduction is allowed for EDX, MTX, and Cis-Pt in a synergistic interaction at a given effect level. Because of the dose reductions that are allowable, the toxicities of each drug are expected to be lower in the combinations.

Although the present investigations can be considered to be small-scale studies, they provide answers to many of the important questions pertaining to the combination of EDX and Cis-Pt. Although the mechanism of these drug interactions are separate issues requiring studies at the biochemical/molecular level, the considerable difference in synergy observed between EDX + Cis-Pt and MTX + Cis-Pt is extremely interesting and will provide additional impetus to pursue such studies at a meaningful level. The other purpose of the present investigations included the illustration of various uses of the methodology of drug-combination data analysis, enabling them to serve as preliminary studies to extend the evaluation of combinations with EDX to other useful antineoplastic agents not only in leukemic cells but also in solid-tumor cell lines.

References

1. Burchenal JH (1978) A clinical overview of dichlorodiammino platinum. Biochimie (Paris) 60: 915–923
2. Chou J (1991) Quantitation of synergism and antagonism of two or more drugs by computerized analysis. In: Chou T-C, Rideout DC (eds) Synergism and antagonism in chemotherapy. Academic Press, New York, pp 223–244
3. Chou J, Chou T-C (1987) Dose-effect analysis with microcomputers: quantitation of ED_{50}, LD_{50}, synergism, antagonism, low-dose risk, receptor-ligand binding and enzyme kinetics. Manual and software, Biosoft, Cambridge, England
4. Chou T-C (1976) Derivation and properties of Michaelis-Menten type and Hill type equations for reference ligands. J Theor Biol 39: 253–276
5. Chou T-C (1991) The median-effect principle and the combination index for quantitation of synergism and antagonism. In: Chou T-C, Rideout D (eds) Synergism and antagonism in chemotherapy. Academic Press, New York, pp 61–102
6. Chou T-C, Talalay P (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
7. Chou T-C, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22: 27–55
8. Chou T-C, Talalay P (1987) Applications of the median-effects principle for the assessment of low-dose risk of carcinogens and for the quantitation of synergism and antagonism of chemotherapeutic agents. In: Harrap KR, Connors TA (eds) New avenues in developmental cancer chemotherapy. (Bristol-Myers Symposium series) Academic Press, New York, pp 37–64
9. Eastman A (1986) Reevaluation of interaction of *cis*-dichloro-(ethylennediammine)platinum(II) with DNA. Biochemistry 25: 3912–3915
10. Kris MG, Kinahan JJ, Gralla RJ, Fanucchi MP, Weitheim MS, O'Connell JP, Marks LD, Williams L, Farag F, Young CW, Sirotnak FM (1988) Phase I trial and clinical pharmacological evaluation of 10-ethyl-10-deazaminopterin in adult patients with advanced cancer. Cancer Res 48: 5573–5579
11. Rozencweig M, Hoff DD von, Abele R, Muggia FM (1980) Cisplatin. In: Pinedo HM (ed), EORTC cancer chemotherapy annual 2. Excerpta Medica, Amsterdam, pp 107–117

12. Schmid FA, Sirotnak FM, Otter GM, et al (1985) New folate analogs of the 10-deazaaminopterin series: markedly increased antitumor activity of the 10-ethyl analog compared to the parent compound and methotrexate against some human tumor xenografts in nude mice. Cancer Treat Rep 69: 551–553
13. Schmid FA, Sirotnak FM, Otter GM, DeGraw JI (1987) Combination chemotherapy with a new folate analog: activity of 10-ethyl-10-deazaminopterin compared to methotrexate with 5-fluorouracil and alkylating agents against advanced metastatic disease in murine tumor models. Cancer Treat Rep 71: 727–732
14. Shum KY, Kris MG, Gralla RJ, Burke MT, Marks LD, Heeland RT (1988) Phase II study of 10-ethyl-10-deaza-aminopterin in patients with stage III and IV nonsmall cell lung cancer. J Clin Oncol 6: 446–450
15. Sirotnak FM, DeGraw JI, Schmid FA, et al (1984) New folate analogs of the 10-deaza-aminopterin series. Further evidence for markedly increased antitumor efficacy compared with methotrexate in ascitic and solid murine tumor models. Cancer Chemother Pharmacol 12: 26–30
16. Sirotnak FM, Schmid FA, Samuels LL, DeGraw JI (1987) 10-Ethyl-10-deaza-aminopterin: structural design and biochemical, pharmacologic, and antitumor properties of folates and folic acid antagonists in cancer chemotherapy. NCI Monogr 5: 127–131
17. Webb JL (1963) Effect of more than one inhibitor. In: Webb JL (ed) Enzyme and metabolic inhibitors, vol 1. Academic Press, New York, pp 66–79, 488–512
18. Zwelling LA, Michaels S, Schwartz H, Dobson OPP, Kohn KW (1981) DNA cross-linking as an indicator of sensitivity and resistance of mouse L1210 leukemia cells to *cis*-diamminedichloroplatinum(II) and L-phenylalanine mustard. Cancer Res 41: 640–649