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Influence of scheduling on two-drug combinations of alkylating agents in vivo*

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Summary. The effects of schedule and sequence on the survival of EMT6 tumor cell and bone marrow (CFU-GM) obtained after treatment using combinations of cyclophosphamide (CTX) and thiotEPA or melphalan (L-PAM) were examined and analyzed by isobologram methodology. On a single-injection schedule, when CTX and thiotEPA were given simultaneously or thiotEPA was given prior to CTX, the result was slightly greater than additive tumor-cell kill. However, when CTX preceded thiotEPA by 4 h, there was less than additive cell kill. When the interval between the administration of the two drugs was 8 h, both sequences of the drugs produced greater than additive tumor-cell kill. Simultaneous administration of CTX and thiotEPA on a multiple-injection schedule resulted in sub-additive tumor-cell kill. On the multiple-injection schedule, extending the interval between injections of CTX and thiotEPA to 4 and 8 h resulted in increasing tumor-cell kill. With the 4- and 8-h intervals, no significant sequence-dependent difference in tumor-cell kill was obtained. The results of CTX and L-PAM combinations paralleled those of CTX and thiotEPA. Bone marrow (CFU-GM) survival was used as a representative normal tissue with which to compare tumor-cell survival after each treatment to obtain a measure of therapeutic effect. The trends for the ratios of bone marrow: tumor cell survival were the same for the treatment sequences of CTX with thiotEPA or L-PAM; however, greater magnitudes of differential tumor-cell kill were obtained with CTX L-PAM combinations. Using this measure, the greatest therapeutic effectiveness was seen with single-dose L-PAM or thiotEPA followed 4 h later by CTX and with CTX given as a single or as multiple doses followed 8 h later by L-PAM or thiotEPA. Such data from tumor-model systems may be useful in the development of more effective alkylating agent regimens for use in the clinic.

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Abbreviations: L-PAM, melphalan, L-phenylalanine mustard; thiotEPA, N,N^{I}N^{II}-triethylenethiophosphoramide; CTX, cyclophosphamide; PBS, phosphate buffered saline; FBS, fetal bovine serum; DME, Dulbecco's minimal essential medium; CFU-GM, granulocyte-macrophage colony forming units.

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Introduction

In developing effective chemotherapeutic regimens using very high drug doses, questions concerning scheduling and sequencing are critical [6, 8, 12, 19–22, 28]. We have previously examined the issue of alkylating agent dose [7, 26] and the effect of scheduling on the therapeutic effect of alkylating agents given singly [27] in tumor-model systems. In this report, we focused on the effect of scheduling on two-drug combinations, specifically, thiotEPA with cyclophosphamide (CTX) and melphalan (L-PAM) with CTX in vivo using EMT6 tumor-cell survival and the granulocyte-macrophage progenitor colony-forming ability of bone marrow as endpoints.

Materials and methods

Drugs. CTX and thiotEPA were obtained from the Dana-Farber Cancer Institute pharmacy. L-PAM was purchased as the pure powder from Sigma Chemical Co. (St. Louis, Mo). For in vivo testing, drugs were freshly prepared just prior to their use, with PBS as the final diluent.

Tumor line. The EMT6 murine mammary carcinoma is an in vivo-in vitro tumor system [15–18]. The tumor was carried in BALB/c mice (Taconic Farms, Germantown, NY). For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of 8- to 10-week-old BALB/c mice.

Tumor excision assay. For each experiment, two tumors were implanted per mouse and two animals were treated at each dose level; therefore, four tumors were pooled at each point. When the tumors had reached approximately 100 mm^3 in volume (about 1 week after tumor-cell implantation), the drugs were given as single doses by i.p. injection (0.2 ml) or as three doses by i.p. injection at 4.5-h intervals over 9 h. Combinations of CTX and thiotEPA or L-PAM were injected i.p. on the various schedules described in Fig. 1–3. Mice were sacrificed 24 h after treatment to enable the full expression of drug cytotoxicity and repair of potentially lethal damage and then soaked in 95% ethanol. The tumors were excised and single-cell suspensions were prepared as previously described [25]. The untreated tumor-cell suspensions had a plating efficiency of 8%–12%. Results are expressed as the surviving fraction (±SE) of cells from treated groups compared

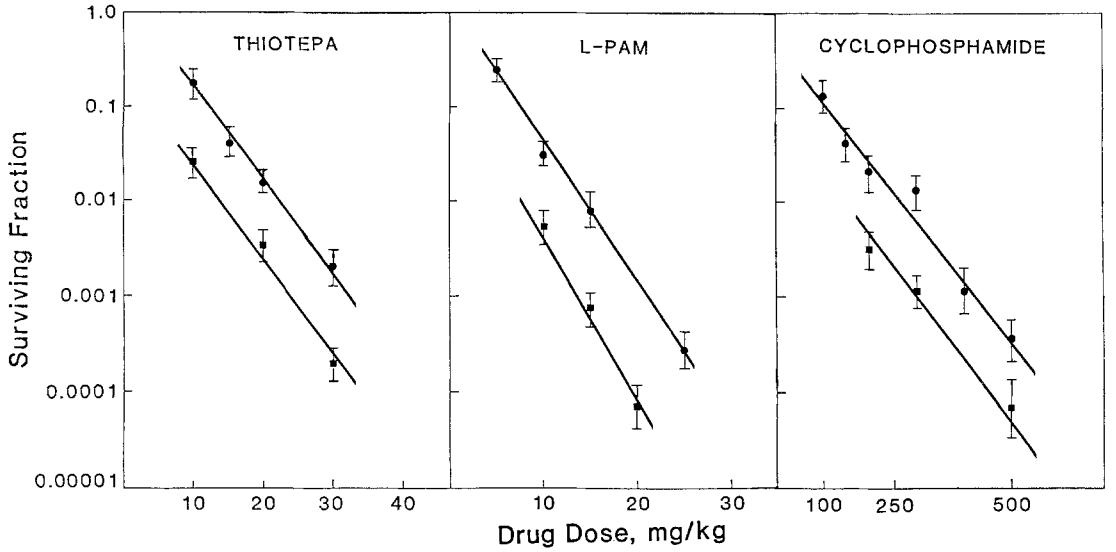

Fig. 1. Survival of EMT6 tumor cells treated in vivo with single doses or with three doses at 4.5-h intervals up to the total dose shown of thiotEPA, L-PAM, or CTX. Single-drug dose (●) and multiple-drug injections to the same total dose (■) for tumor cells. Data points represent the mean of three independent determinations; bars represent the SE

with untreated control values from three independent experiments.

Bone marrow toxicity. Bone marrow was taken from the same animals used for the tumor excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle, and CFU-GM assay was carried out as previously described [25]. Colonies of at least 50 cells were scored on an Acculite colony counter (Fisher Scientific, Springfield, NJ). The results from three experiments, in which each group was measured in duplicate at three cell concentrations, were averaged. The results are expressed as the surviving fraction of treated groups compared with that of untreated controls.

Data analysis. Quantitative analysis of survival curves was carried out using the log-probit iterative least-squares method of Litchfield and Wilcoxon [11] as revised by Tallarida and Murray [24]. Calculations were done on an Apple IIC microcomputer. The method of Deen and Williams [4] was used to generate isobolograms for the special case in which the dose of one agent is held constant. This method produces envelopes of additive effect for different levels of the variable agent. It is conceptually identical to generating a series of isobolograms and replotting the results at a constant dose of one agent on a log effect by dose of the second agent in a coordinate system. Dose-response curves were first generated for each individual agent. The envelopes of additivity shown in Figs. 1–3 were generated from a series of iso-effect curves derived from the complete dose-response curves for each agent.

Overall, combinations producing the desired effect that are within the envelope boundaries of modes I and II are considered to be additive. Those displaced to the left are supra-additive and those displaced to the right are sub-additive [2, 23]. This general approach can be extrapolated to the special case in which the level of an agent is held constant. Under these conditions, an isobologram can be derived that plots the expected effect (modes I and II) for any level of the variable agent plus the constant-agent combinations [5]. Experimentally, this approach is far simpler and readily facilitates the determination of additive and nonadditive combinations.

To facilitate these analyses, a flexible, interactive computer program was written in BASIC for the Apple II+ microcomputer. The program first derives the best-fitting dose-response curves using dose or log dose and effect, log effect, probit percentage of effect, or logit percentage of effect relations; for cell-survival dose-response curves, correlations of $\geqslant 0.96$ have been obtained. The program then calculates an isobologram at a constant level of the selected agent and plots the data.

Results

Tumor-cell survival curves for thiotEPA, L-PAM, and CTX given as single bolus injections or as three injections over 9 h to the same total dose are shown in Fig. 1. When thiotEPA was injected on the multiple-dose schedule, there was an increase in tumor-cell kill of 7- to 10-fold over the dose range of the drug examined; when L-PAM was given as three injections, its toxicity to tumor cells also increased relative to that obtained with single-dose administration. The increase in tumor-cell kill by L-PAM ranged from 8- to 15-fold over the dose range examined. Administration of CTX on the multiple-injection schedule resulted in a 5- to 6-fold increase in tumor-cell kill over the dose range examined, compared with the same total CTX dose given as a single injection. The maximal tolerated doses of each of the drugs given as single i. p. injections are approximately 10 mg/kg for thiotEPA and L-PAM and about 200 mg/kg for CTX. To obtain tumor-cell survival data through several logs, animals were treated with doses of each of the three drugs that would be lethal at times of >24 h posttreatment. These single-agent survival curves were used to generate envelopes of additivity for the two drug combinations.

The effects of schedule and sequence on EMT6 tumor cell kill obtained with combinations of CTX and thiotEPA

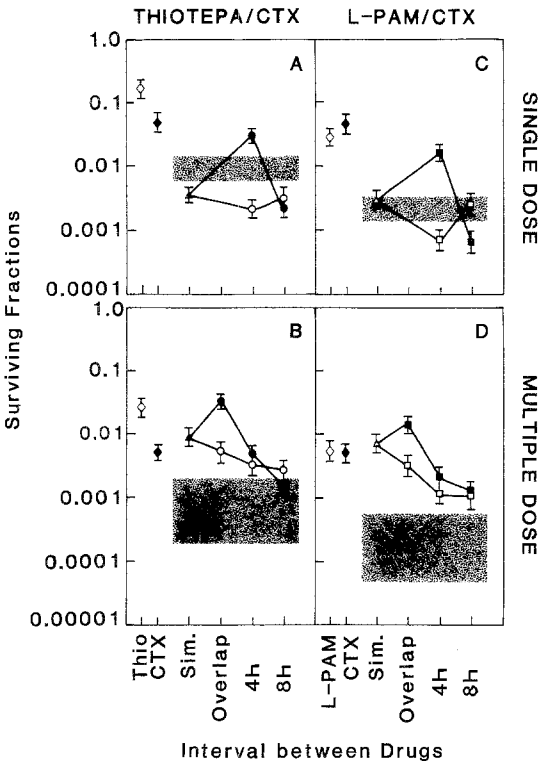

Fig. 2. Survival of EMT6 tumor cells treated in vivo with CTX alone or in combination with thiotEPA or L-PAM in various schedules and sequences. CTX was given as a single dose of 150 mg/kg or as three injections of 50 mg/kg at 4.5-h intervals. ThiotEPA and L-PAM were given as single doses of 10 mg/kg or as three injections of 3.3 mg/kg at 4.5-h intervals. Survival after exposure to the single agents is shown as (◇, ◆); survival after the drug combinations were given simultaneously is shown as (▲, △); survival after sequences in which CTX was given first is shown as (●, ■); survival after sequences in which either thiotEPA or L-PAM was given first is shown as (○, □). Overlap indicates that thiotEPA or L-PAM was given with the first (●, ■) or last (○, □) injection of CTX. The 4- and 8-h intervals were measured from the last drug injection of the first agent on the multiple-dose schedules. The shaded area in each panel indicates the envelope of additivity for tumor-cell killing of the two drugs as determined by isobologram analysis of the survival curves for each agent given as single or multiple doses. Data points represent the mean of three independent determinations; bars represent the SE

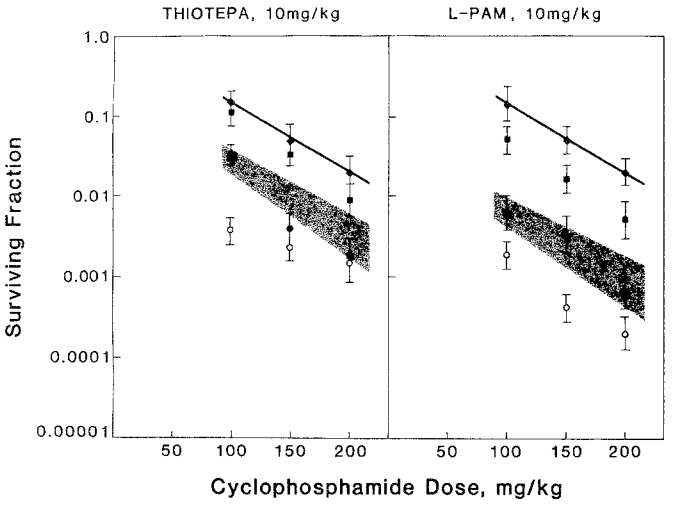

Fig. 3. Survival curves for EMT6 tumor cells treated in vivo with 10 mg/kg thiotEPA or L-PAM and various doses of CTX on different schedules. The upper line (◆) represents the survival of EMT6 cells exposed to single doses of CTX only. Survival after simultaneous exposure to single doses of CTX and thiotEPA or L-PAM is shown as (●); CTX followed 4 h later by thiotEPA or L-PAM is shown as (■); thiotEPA or L-PAM followed 4 h later by CTX is shown as (○). Shaded areas indicate the envelopes of additivity determined by isobologram analysis of the survival curves for each drug combination. Data points represent the mean of three independent determinations; bars represent the SE

or L-PAM were examined. As shown in Fig. 2, CTX was given either as a single injection of 150 mg/kg or as three injections of 50 mg/kg at 4.5-h intervals. The shaded areas in Fig. 2 indicate the envelopes of additivity for the combination of CTX (150 mg/kg) and either thiotEPA (10 mg/kg) or L-PAM (10 mg/kg), obtained from isobologram analysis [2, 4, 5, 23, 26]. The level of tumor-cell kill obtained with two drugs in combination varied, depending on the schedule and sequence of drug administration. CTX and thiotEPA given simultaneously in single injections resulted in a slightly greater than additive cell kill (Fig. 2, panel *A*). When the drugs were injected with a 4-h interval between them and thiotEPA was given first, an additional 2-fold increase in tumor-cell kill resulted. However, when CTX preceded thiotEPA by 4 h, there was less than additive cell kill and 1 log less cell kill than that obtained when thiotEPA was given first. On the other hand, when the interval between the administration of the two drugs was 8 h, both drug sequences produced greater than additive tumor-cell kill that was the same as that obtained with thiotEPA followed 4 h later by CTX.

Figure 2 (panel *B*) shows the envelope of additivity resulting from the combination of thiotEPA and CTX given on the multiple-injection schedule. Simultaneous administration of both drugs on the multiple-injection schedule gave subadditive tumor-cell kill. The initial administration of thiotEPA as a single injection followed by CTX given on the multiple-injection schedule was, again, less than additive; however, when thiotEPA was given as a single injection after the administration of CTX on the multiple-dose schedule, the combination was slightly antagonistic. The observed cell kill was actually slightly less than that obtained with either single agent given as three injections at 4.5-h intervals. Extending the interval between CTX and thiotEPA injections to 4 and then 8 h resulted in increasing tumor-cell kill. With the 4- and 8-h intervals, no significant sequence-dependent difference in tumor-cell kill was obtained.

The results of CTX and L-PAM combinations paralleled those of CTX and thiotEPA. When CTX and L-PAM were given simultaneously as single injections, the effect was additive (Fig. 2, panel *C*). The tumor-cell kill obtained when L-PAM was injected as a single dose 4 h prior to administration of CTX as a single dose was greater than additive. However, when single-dose CTX preceded L-PAM, the tumor-cell kill was less than additive. On the other hand, when the interval between drugs was extended to 8 h, the sequence of CTX followed by L-PAM resulted in greater than additive cell kill, whereas the opposite sequence (L-PAM → CTX) was additive.

Table 1. Ratios of bone marrow (CFU-GM) survival to EMT6 tumor-cell survival in mice treated with cyclophosphamide and thiotEPA or melphalan[a]

Treatment sequence	Ratio (Bone marrow/tumor) interval between drugs		
	0 h	4 h	8 h
CTX(sd)/Thio(sd)[b]	10.3	12.8	15.5
Thio(sd)/CTX(sd)	10.3	13.0	6.7
CTX(md)/Thio(sd)	1.4	5.5	11.2
Thio(sd)/CTX(md)	9.6	11.8	10.4
CTX(md)/Thio(md)	10.8	–	–
CTX(sd)/L-PAM(sd)	7.1	10.6	74.0
L-PAM(sd)/CTX(sd)	7.1	107.0	19.6
CTX(md)/L-PAM(sd)	5.5	46.4	78.3
L-PAM(sd)/CTX(md)	44.4	61.7	39.1
CTX(md)/L-PAM(md)	12.3	–	–

[a] The ratios for the single-dose agents are: CTX(sd), single dose (150 mg/kg) 1.6; CTX(md), multiple dose (3 × 50 mg/kg) 80.0; Thio(sd), single dose (10 mg/kg) 0.21; Thio(md), multiple dose (3 × 3.3 mg/kg) 1.9; L-PAM(sd), single dose (10 mg/kg) 5.7; L-PAM(md), multiple dose (10 mg/kg) 3.1

[b] Treatment groups correspond to those shown in Fig. 1

The envelope of additivity for the combination of L-PAM and CTX given on the multiple-injection schedule is shown in Fig. 2 (panel *D*). Simultaneous administration of both drugs on the multiple-dose schedule resulted in much less than additive tumor-cell kill. When L-PAM was given first as a single injection followed by CTX given on the multiple-dose schedule, the cell kill was, again, less than additive. However, when L-PAM was given as a single injection following CTX given on the multiple-dose schedule, the resultant cell kill was less than that obtained using either single agent given as three injections 4.5-h apart. As the interval between the drugs was increased to 4 and then to 8 h, an increasing tumor-cell kill was obtained with CTX followed by L-PAM and, to a smaller degree, with the opposite treatment sequence; however, in all cases the tumor-cell kill obtained was less than expected for additivity of the combination.

Three sequences in which drugs were delivered as single injections were examined over a CTX dose range (Fig. 3). Initial administration of CTX followed 4 h later by either thiotEPA or L-PAM substantially negated the effect of the second drug, resulting in less than additive tumor-cell kill over the dose range with both thiotEPA and L-PAM. Simultaneous administration of either CTX and thiotEPA or CTX and L-PAM produced essentially additive cell kill over the CTX dose range examined. When either thiotEPA or L-PAM preceded the administration of CTX, greater than additive tumor-cell kill was observed over the CTX dose range. With thiotEPA, the degree of supra-additivity decreased with increasing CTX dose, such that at the highest CTX dose there was no difference in tumor-cell kill between the simultaneous drug schedule and the thiotEPA→CTX sequential schedule. The L-PAM→CTX sequential schedule resulted in a significant increase in tumor-cell kill relative to that achieved with simultaneous drug administration; this increase persisted over the CTX dose range tested.

For the drug-combination studies, bone marrow (CFU-GM) survival was used as a representative (normal tissue) with which to compare tumor-cell survival after each treatment to obtain a measure of therapeutic effect. The level of bone marrow kill by all of these various treatment combinations was in the range of 1–1.5 logs; therefore, the variation in the ratios of bone marrow: tumor cell survival are due mainly to differences in the tumor-cell kill produced by the various treatment combinations. Ratios for the bone marrow: tumor cell survival for the treatments shown in Fig. 1 are given in Table 1. The trends were the same for the treatment sequences of CTX with thiotEPA or L-PAM; however, greater magnitudes of differential tumor-cell kill were obtained with CTX/L-PAM combinations. When CTX, whether as a single dose or as multiple doses, was first in the treatment sequence, followed by either thiotEPA or L-PAM, the differential between bone marrow and tumor-cell kill increased with increasing intervals between drugs. The bone marrow: tumor-cell survival ratio for single-dose CTX combined with single-dose thiotEPA increased from 10.3 (simultaneous administration) to 15.5 (8-h interval between drugs). Similarly, for single-dose CTX combined with single-dose L-PAM, the bone marrow: tumor-cell survival ratio increased from 7.1 (simultaneous administration) to 74.0 (8-h interval between drugs). The same pattern was observed when CTX was given on the multiple-dose schedule. When thiotEPA was injected with the last dose of CTX, this survival ratio was 1.4, which increased to 11.2 when thiotEPA was given 8 h after the final CTX injection. With multiple-dose CTX, when L-PAM was given simultaneously with the last CTX injection, a bone marrow: tumor-cell survival ratio of 5.5 was obtained. Extending the interval between the last dose of CTX and the administration of L-PAM to 8 h increased this survival ratio to 78.3.

When thiotEPA or L-PAM were given first, the 4-h interval produced the greatest differentials between bone marrow and tumor-cell kill, whether CTX was given as a single dose or as multiple injections. The effect of the intervals between drug administration on the ratio of bone marrow: tumor cell survival was not very large with the treatment sequence of thiotEPA followed by CTX; however, when the drugs were L-PAM and CTX, large differences in this survival ratio were observed. The greatest differential (107.0) between bone marrow and tumor-cell survival was seen when a single dose of L-PAM was followed 4 h later by a single dose of CTX. L-PAM followed by CTX on the multiple-dose schedule resulted in a maximal bone marrow: tumor-cell survival ratio of 61.70, which occurred when a single dose of L-PAM was followed 4 h later by CTX on the multiple-dose schedule.

Discussion

It has long been recognized that the schedule and sequence of drugs in combination regimens can affect therapeutic outcome. Over the last 15 years, the definition of additivity and therapeutic synergism has evolved with increasing stringency. In the work of Schabel et al. [19–21], therapeutic synergism between two drugs was defined to mean that "the effect of the two drugs in combination was significantly greater than that which could be obtained when either drug was used alone under identical condi-

tions of treatment". Using this definition, the combination of CTX and L-PAM given simultaneously by i.p. injection every 2 weeks was reported to be therapeutically synergistic in the Ridgeway osteosarcoma growth-delay assay [19–21]. Similarly, the combination of CTX and L-PAM has been reported to be therapeutically synergistic in L1210 and P388 leukemias [21]. CTX plus a nitrosourea (BCNU, CCNU, or MeCCNU) have also been reported to be therapeutically synergistic in increase-in-life-span and growth-delay assays using the above definition [21].

The availability of quantitative methods for the measurement of tumor-cell kill in vitro and from in vivo treatments enables the application of more rigorous definitions of synergy (supra-additivity) and additivity to anticancer drug-treatment data. Figures 2 and 3 show envelopes of additivity generated from isobolograms derived from the survival curves for each drug given alone as single or multiple doses. Using this definition of additivity in Fig. 2, of the various schedules combining CTX (total dose, 150 mg/kg) and thiotEPA (total dose, 10 mg/kg), only the single-dose regimen with thiotEPA given before or with CTX was consistently synergistic.

With the combination of CTX (total dose, 150 mg/kg) and L-PAM (total dose 10 mg/kg), synergy was obtained with L-PAM followed 4 h later by CTX and with CTX followed 8 h later by L-PAM. The other schedules of CTX and L-PAM at these doses were additive or subadditive. However, when the interval between drug administration was 8 h, drug sequence did not affect the level of tumor-cell kill obtained with the single-dose regimen, and both sequences resulted in greater than additive cell kill.

Because there was greater tumor-cell kill with both CTX and thiotEPA given as single agents using the multiple-dose regimen, the envelope of additivity for these drugs on that schedule indicates greater tumor-cell kill than for the same drugs on the single-injection schedule. In fact, the cell kill observed with CTX (3×50 mg/kg; total dose, 150 mg/kg) and thiotEPA (total dose, 10 mg/kg) on the four multiple-dose protocols was in the same range as that seen with the single-dose regimens, and only one point [CTX (3×50 mg/kg) followed 8 h later by thiotEPA (10 mg/kg)] was within the envelope of additivity. Given as multiple doses, most schedules of CTX and thiotEPA and all tested schedules of CTX and L-PAM led to less than additive killing of tumor cells as determined by isobologram analysis of the tumor-cell kill produced by the drugs as single agents. Perhaps of most concern is the observation that CTX given shortly prior to either thiotEPA or L-PAM produced less tumor-cell kill than when the drugs were given as single injections and when CTX was either given simultaneously with thiotEPA or L-PAM or injected first in combination with thiotEPA or L-PAM, it produced less tumor-cell kill than when given alone.

In agreement with our findings, Hagenbeek and Martens [9] found in the rat BNML leukemia model that the sequence of total-body irradiation followed by CTX produced greater tumor-cell kill than did CTX followed by total-body irradiation. Similarly, when the combination of busulfan and CTX was tested in the BNML model, the sequence of busulfan followed by CTX produced greater tumor-cell kill than did CTX followed by busulfan. It is certainly possible that there are more effective schedules for combinations of CTX and thiotEPA or L-PAM than those presented in Fig. 2. ThiotEPA and L-PAM are relatively short-lived in circulation, whereas CTX is relatively long-lived, with formation of the active species over the course of several hours ($t_{1/2}$ 6–7 h) [3].

It appears that the presence of CTX and/or its metabolites in circulation and/or the tumor masks or interferes with the cytotoxic action of thiotEPA and L-PAM, since separating the drugs in time seems to result in greater tumor-cell kill than simultaneous administration or short-interval separation of the drugs; thus, for these particular drug combinations there appears to be an advantage in allowing one drug to be in large part cleared from the circulation before the second drug is given. As shown in Fig. 3, over a CTX dose range, drug combinations can be subadditive, additive, or supra-additive, depending on the sequence of administration.

Although the range of tumor-cell kill obtained with these alkylating agent combinations varied more than 10-fold (depending on the schedule and sequence of administration), with the dose held constant, the data tend to indicate that an approximately 3-log tumor-cell kill is about the maximum obtainable with these drugs at these doses in this murine tumor model. This maximal tumor-cell kill may be a reflection of many factors, such as the ability of these agents to penetrate the tumor mass, or of the occurrence of resistant cellular subpopulations within the tumor. At this maximal level of tumor-cell kill for a given drug combination and dose, the schedule and sequence are no longer important.

There was less variation in bone marrow (CFU-GM) kill with the CTX and thiotEPA or L-PAM combinations than there was in tumor-cell kill with these treatments. A comparison of bone marrow and tumor-cell survival, made in an effort to develop an indication of therapeutic effectiveness after treatment with CTX and thiotEPA or L-PAM in various scheduls and sequences, revealed that trends in the ratios of bone marrow: tumor survival were the same, whether the agent used in combination with CTX was thiotEPA or L-PAM. The largest variations in these ratios were observed with the combination of CTX and L-PAM; using this measure, the greatest therapeutic effectiveness was seen with single-dose L-PAM followed 4 h later by CTX and with CTX given as a single dose or as multiple doses followed 8 h later by L-PAM. These particular schedules and sequences for CTX and L-PAM also correspond to points of maximal tumor-cell kill.

Each of these drugs is known to reduce the glutathione content of cells in culture [1, 10, 13, 14]. Millar and others [10, 13, 14] observed that a low "priming" dose of CTX given prior to a higher therapeutic dose resulted in less toxicity of the therapeutic dose to the bone marrow. This concept is currently being clinically applied in patients receiving high-dose chemotherapy with CTX or L-PAM [10]. Adams et al. [1] examined the effect of high-dose CTX (500 mg/kg) on the glutathione levels and glutathione-S-transferase activities in bone marrow over the course of time. The nadirs for glutathione content and glutathione-S-transferase activity in bone marrow were reached approximately 2 days after CTX administration. The level of glutathione-S-transferase activity subsequently increased, reaching a peak at about 5 days after CTX administration. Whether early changes in glutathione and/or glutathione-S-transferase levels are involved in the variations in tumor cell and bone marrow survival observed in the present studies is not known.

In conclusion, there clearly appear to be less effective ways of giving CTX with thiotEPA or L-PAM, and there appears to be a variety of more effective ways to treat tumors with the same drug combinations. The knowledge of such information from model systems may be instrumental in the development of more effective alkylating agent combinations for use in the clinic.

References

1. Adams DJ, Carmichael J, Wolf CR (1985) Altered mouse bone marrow glutathione and glutathione transferase levels in response to cytotoxins. Cancer Res 45: 1669
2. Berenbaum MC (1977) Synergy, additivism and antagonism in immunosuppression. Clin Exp Immunol 28: 1
3. Colvin M (1982) The alkylating agents. In: Pharmacologic principles of cancer treatment. Saunders, Philadelphia, pp 276–306
4. Deen DF, Williams MW (1979) Isobologram analysis of X-ray-BCNU interactions in vitro. Radiat Res 79: 483
5. Dewey WC, Stone LE, Miller HH, Giblak RE (1971) Radiosensitization with 5-bromodeoxyuridine of Chinese hamster cells X-irradiated during different phases of the cell cycle. Radiat Res 47: 672
6. Frei E III (1985) Curative cancer chemotherapy. Cancer Res 45: 6523–6537
7. Frei E III, Teicher BA, Holden SA, Cathcart KNS, Wang Y (1988) Effect of alkylating agent dose: preclinical studies and possible clinical correlation. Cancer Res 48: (in press)
8. Goldin A (1984) Dosing and sequencing for antineoplastic synergism in combination chemotherapy. Cancer 54: 1155
9. Hagenbeek A, Martens AC (1987) Conditioning regimens before bone marrow transplantation in acute myelocytic leukemia. In: Autologous bone marrow transplantation. The University of Texas, Houston, pp 99–103
10. Hedley DW, McElwain EJ, Millar JL, Gordon MY (1978) Acceleration of bone marrow recovery by pretreatment with cyclophosphamide in patients receiving high-dose melphalan. Lancet II: 966
11. Litchfield JT, Wilcoxon FA (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96: 99
12. Mihich E, Grindey GB (1977) Multiple basis of combination chemotherapy. Cancer 40: 534
13. Millar JL, Hudspith BN, Blackett NM (1975) Reduced lethality in mice receiving a combined dose of cyclophosphamide and busulphan. Br J Cancer 32: 193
14. Millar JL, Blackett NM, Hudspith BN (1978) Enhanced post-irradiation recovery of the haemopoietic system in animals pretreated with a variety of cytotoxic agents. Cell Tissue Kinet 11: 543
15. Rockwell S (1977) In vivo-in vitro tumor systems: new models for studying the response of tumors in therapy. Lab Anim Sci 27: 831
16. Rockwell S (1978) Cytotoxic and radiosensitizing effects of hypoxic cell sensitizers on EMT6 mouse mammary tumor cells in vivo and in vitro. Br J Cancer 37: 212
17. Rockwell S, Kallman RF (1973) Cellular radiosensitivity and tumor radiation response in the EMT6 tumor cell system. Radiat Res 53: 281
18. Rockwell S, Kallman RF, Fajardo LF (1972) Characteristics of a serially transplanted mouse mammary tumor and its tissue culture adaption. J Natl Cancer Inst 49: 735
19. Schabel FM, Trader MW, Laster WR, Wheeler GP, Witt MH (1978) Patterns of resistance and therapeutic synergism among alkylating agents. Antibiotic Chemother 23: 200
20. Schabel FM, Griswold DP, Corbett TH, Laster WR, Mayo JG, Lloyd HH (1979) Testing therapeutic hypotheses in mice and man: observations on the therapeutic activity against advanced solid tumors of mice treated with anticancer drugs that have demonstrated or potential clinical utility for treatment of advanced solid tumors of man. Methods Cancer Res 17: 1
21. Schabel FM, Griswold DP, Coorbett TH, Laster WR (1983) Increasing therapeutic response rates to anticancer drugs by applying the basic principles of pharmacology. Pharmacol Ther 20: 283
22. Skipper HE (1974) Combination therapy: some concepts and results. Cancer Chemother Rep 4: 137
23. Steel GG, Peckham MJ (1979) Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat FQ
24. Tallarida RJ, Murray RB (1981) Manual of pharmacologic calculations with computer programs. Springer, New York
25. Teicher BA, Holden SA, Jacobs JL (1987) Approaches to defining the mechanism of enhancement by fluosol-DA 20% with carbogen of melphalan antitumor activity. Cancer Res 47: 513
26. Teicher BA, Holden SA, Cucchi CA, Cathcart KNS, Korbut TT, Flatow JL, Frei E III (1988) Combination of *N,N',N''*-triethylenephosphoramide and cyclophosphamide in vitro and in vivo. Cancer Res 48: 94
27. Teicher BA, Holden SA, Eder JP, Brann TW, Jones SM, Frei E III (1989) Influence of scheduling on alkylating agent cytotoxicity in vitro and in vivo. Cancer Res (in press)
28. Wittes RE, Goldin A (1986) Unresolved issues in combination chemotherapy. Cancer Treat Rep 70: 105

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