Cancer Chemother Pharmacol (1995) 36: 61–64

ORIGINAL PAPER

Sylvia A. Holden · Beverly A. Teicher · Lois J. Ayash
Emil Frei III

A preclinical model for sequential high-dose chemotherapy

Received: 23 March 1994 / Accepted: 22 September 1994

Abstract Dose-intensive chemotherapy regimens have entered clinical trial based on the notion that log-linear tumor-cell killing, especially with antitumor alkylating agents, is maintained at higher drug doses. Several clinical trials employing two intensifications are underway. Using the tumor-cell survival assay, animals bearing the FSaII fibrosarcoma were treated with single doses of various chemotherapeutic agents once or twice with a 3- or 7-day interval between the drugs. Isobologram methodology was used to determine if the sequential treatment regimens resulted in subadditive, additive or greater-than-additive tumor-cell killing. When melphalan was followed 3 or 7 days later by a second dose of melphalan there was evidence of resistance to the second dose of melphalan as indicated by subadditive tumor-cell killing. Melphalan followed 3 days later by cyclophosphamide (300 mg/kg) produced greater-than-additive tumor-cell killing, however, when the interval was 7 days the resulting tumor-cell killing was subadditive. Melphalan followed 3 or 7 days later by thiotepa or carboplatin produced subadditive-to-additive tumor-cell killing. Adriamycin followed 3 days later by melphalan, cyclophosphamide, thiotepa, or carboplatin resulted in subadditive-to-additive tumor-cell killing by the combinations. These results indicate that sequential drug-intensive treatments may not optimize tumor-cell killing in vivo.

Key words Sequential high-dose chemotherapy
Antitumor alkylating agents · Tumor-cell killing

Introduction

The necessity of eradicating through therapeutic intervention nearly all of the tumor-cells in a host to achieve tumor cure has been well established in both preclinical and clinical studies [2, 3, 9]. As evident disease comprises many powers of 10 of cells, tumor-cell killing is often described in logs. The two major variables in the administration of chemotherapeutic agents to achieve maximal logs of tumor-cell killing are the dose and schedule. The schedule is critical, especially with cell-cycle-specific agents, to the continuation of an effect on a drug-sensitive tumor-cell subpopulation. Many anticancer agents kill tumor-cells in a log-linear manner with increasing dose or concentration of the agent. The major variable that characterizes the response of tumor-cells to agents that kill in a log-linear manner is the slope of the tumor-cell survival curve. For cells that are very sensitive to an agent the slope of the survival curve is steep, such that many more logs of cells are killed by small increases in drug dose or concentration. For cells that are resistant to an agent the slope of the survival curve is shallow, such that increases in drug dose or concentration produce only modest increases in tumor-cell killing [2, 3, 9]. Given these characteristics of cancer chemotherapy, perhaps the factors most important to the treatment outcome are the tumor-cell burden and tumor-cell sensitivity/resistance to the cytotoxic agent.

The advent of bone marrow/peripheral blood stem-cell supportive care allows the issues of dose and tumor burden to be explored in the clinic to previously unattainable levels [2, 3]. In the current report, we examine the effects on tumor-cell killing of doses of antitumor agents given 3 or 7 days apart to animals bearing the FSaII fibrosarcoma in an effort to model double dose-intensive chemotherapy regimens.

Materials and methods

Drugs

Melphalan (L-phenylalanine mustard, L-PAM) and cyclophosphamide (CTX) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Thiotepa was a gift from Lederle Laboratories (Pearl River, N.Y.). All

This work was supported by NIH grant PO1ßCA38493

S.A. Holden · B.A. Teicher (✉) · L.J. Ayash · E. Frei III

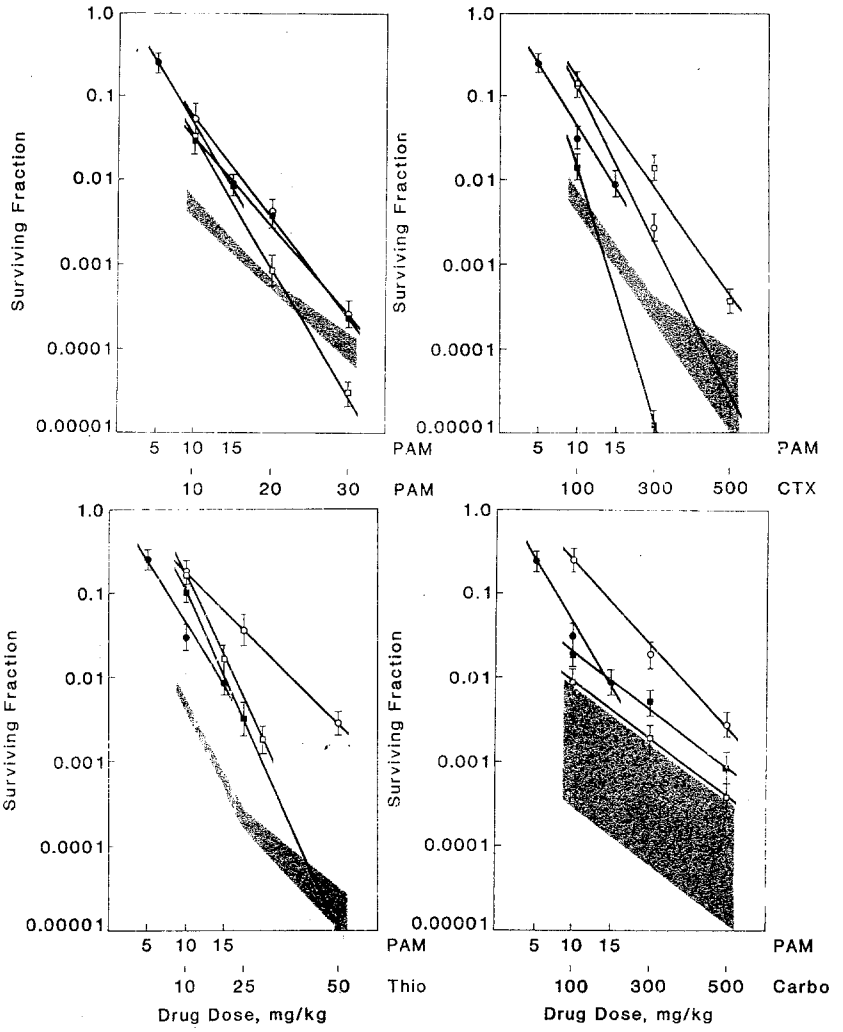

Fig. 1 Survival of FSaII tumor-cells from tumors treated in vivo with 10 mg/kg melphalan (●) followed 3 days later (■) or 7 days later (O) by various doses of a second antitumor alkylating agent. The *shaded areas* are the envelopes of additivity as determined by isobologram analysis. The second alkylating agent alone is shown as (❑). Each experiment was repeated three times. *Points* represent mean values; *bars* indicate SEM

other drugs were purchased from the Dana-Farber Cancer Institute pharmacy.

Tumor

The FSaII fibrosarcoma [7] adapted for growth in culture (FSaIIC) [1, 10] was carried in male C3H/He mice (Jackson Laboratory, Bar Harbor, Me.). For the experiments, 2×10^6 tumor-cells prepared from a brei of several stock tumors were implanted s.c. into the legs of male mice aged 8–10 weeks.

Tumor-excision assay

When the tumors had reached a volume of approximately 100 mm^3 (about 1 week after tumor-cell implantation), the animals were injected i.p. with various single doses of each of the drugs alone [melphalan (5, 10, or 15 mg/kg), cyclophosphamide (100, 300, or 500 mg/kg), thiotepa (10, 25, or 50 mg/kg), carboplatin (100, 300, or 500 mg/kg), or Adriamycin (5, 10, or 15 mg/kg)] or were injected i.p. with melphalan (10 mg/kg) or Adriamycin (10 mg/kg) followed 3 or 7 days later by various doses of each drug. Mice were killed at 24 h after treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage. The tumors were excised and single-cell suspensions were prepared as previously described [10, 11]. The plating efficiency of untreated tumor-cell suspensions ranged from 10% to 16%. The results are expressed as the surviving fraction (± SE) of cells from treated groups as compared with untreated controls [11].

Results

Intensification regimens often employ antitumor alkylating agents because bone marrow or peripheral blood-stem cell reinfusion has allowed previously lethal doses of these drugs to be given to patients safely [2, 3]. Each of the antitumor alkylating agents injected into tumor-bearing animals killed FSaIIC tumor-cells in a log-linear manner with increasing dose of each drug (Fig. 1). The tumor-cell killing obtained when melphalan was injected first followed 3 or 7 days later by a second dose of cyclophosphamide, thiotepa, or carboplatin is shown in Fig. 1. When melphalan (10 mg/kg) was given to the tumor-bearing animals followed 3 or 7 days later by various doses of melphalan, some evidence existed for tumor-cell resistance to the second dose of melphalan as indicated by the shallow slope of the tumor-cell killing curve on both days 3 and 7 after the first exposure to the drug. At a dose of 30 mg/kg melphalan the diminution in cell killing in the two-dose protocol was nearly 1 log as compared with one high-dose melphalan treatment.

In contrast, if a dose of 10 mg/kg melphalan was followed 3 days later by various doses of cyclophospha-

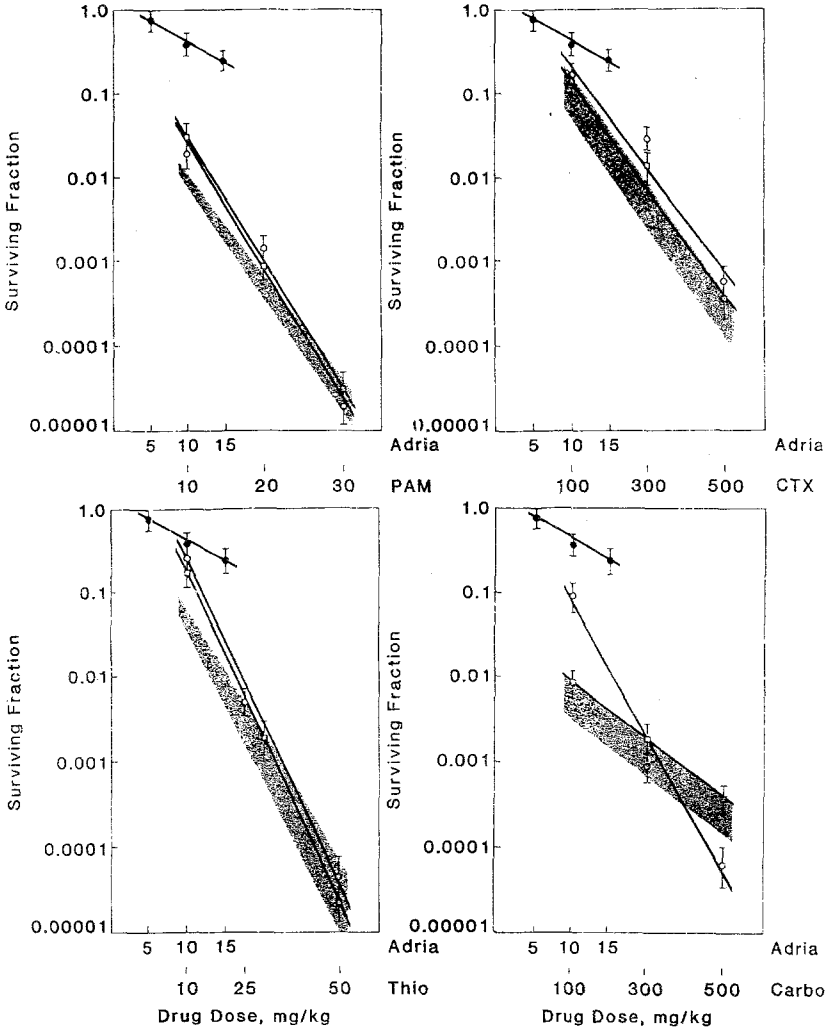

Fig. 2 Survival of FSaII tumor-cells from tumors treated in vivo with Adriamycin (10 mg/kg) (●) followed 3 days later (O) by various doses of an antitumor alkylating agent. The *shaded areas* are the envelopes of additivity as determined by isobologram analysis. The alkylating agent alone is shown as (❑). Each experiment was repeated three times. *Points* represent mean values; *bars* indicated SEM

mide, greater-than-additive tumor-cell killing was obtained with 300 mg/kg cyclophosphamide. If the interval between the drugs was extended to 7 days, the treatment combination resulted in subadditive-to-additive tumor-cell killing. When a dose of 10 mg/kg melphalan was followed 3 days later by various doses of thiotepa the tumor-cell killing of the combination was subadditive except at the highest dose of thiotepa, where additivity was achieved. If the interval between the drugs was extended to 7 days, antagonism was evident between the two drugs that increased in degree at higher thiotepa doses. The combination of melphalan and carboplatin also produced a complex pattern of response. When the drugs were separated by a 3-day interval, antagonism resulted; the degree of antagonism was much greater, however, when the interval between drug exposures was increased to 7 days.

The FSaII fibrosarcoma is not very sensitive to the cytotoxicity of Adriamycin (Fig. 2). A dose of 10 mg/kg Adriamycin kills 64% of the tumor-cells (surviving fraction, 0.36). Administration of 10 mg/kg Adriamycin 3 days prior to various doses of melphalan, cyclophosphamide, or thiotepa did not alter the tumor-cell killing from that obtained with each of the antitumor alkylating agents alone. When FSaIIC tumor-bearing animals were treated with 10 mg/kg Adriamycin followed 3 days later by various doses of carboplatin the slope of the tumor-cell survival curve was altered such that at a standard dose (100 mg/kg) of carboplatin the combination was subadditive but at a high carboplatin dose (500 mg/kg) the combination was greater-than-additive.

Discussion

As the knowledge of anticancer drug:drug and drug:target interactions has increased as well as the realization that measurable drug resistance can be induced in tumor-cells after, in some cases, only one exposure to the agent, the appreciation for the importance of drug scheduling both for individual agents and relative to other agents in a regimen has grown. For example, the achievement of greater-than-additive cytotoxicity between a topoisomerase II inhibitor and an antitumor alkylating agent requires that the topoisomerase II inhibitor be in place at the time of alkylating agent exposure, and reversal at the multidrug resistance

(MDR) phenotype requires that the agent being utilized to block the efflux pump be present during exposure to the more susceptible drug. In the development of maximally cytotoxic combinations of antitumor alkylating agents, pharmacokinetics and drug-metabolism issues may be important. In previous studies [2, 12], the two-drug combinations of thiotepa or melphalan with cyclophosphamide were examined in 24 schedules delivered over an 8-h period to the same total dose. Of these, 6 schedules resulted in greater-than-additive cell killing and 15 resulted in subadditive tumor-cell killing. The generalizations that emerged from these studies were that dividing a total dose into fractions over an 8-h period resulted in less tumor-cell killing and administration of cyclophosphamide first in the sequence resulted in less tumor-cell killing.

The current study explored the use of longer intervals between two drug combinations with either melphalan or Adriamycin being given first. Exposure to melphalan, more than that to other antitumor alkylating agents, appears to induce resistance to itself rapidly and, frequently, cross-resistance to other alkylating agents in established melphalan-resistant cell lines [4–6, 8]. Upon treatment of tumor-bearing animals with melphalan followed 3 or 7 days later by a second dose of melphalan, resistance was evident at 3 or more logs of cell killing. This finding is very similar to that seen with MCF-7 in cell culture using 1 or 7 days between exposures to melphalan [6]. The doubling time of the untreated FSaIIC fibrosarcoma tumors is about 2.5 days and the generation time of MCF-7 cells in culture is about 24 h; therefore, the shorter intervals between the exposures to melphalan (3 days in vivo and 24 h in vitro allowed 0 or 1 cell doubling between treatments and may reflect induction of resistance in the same cells that survived the initial melphalan exposure. The 7-day interval between melphalan exposures may reflect the response primarily of the daughter cells of the original FSaIIC or MCF-7 populations, indicating that this resistance can persist for at least a few generations. Therefore, the growth fraction of the tumor at the time of treatment may be important in the cell killing obtained from drug treatment.

Although resistance to thiotepa and carboplatin was not evident (to a major degree) when the melphalan administration occurred 3 days prior to treatment with the second drug, with a 7-day interval, resistance expressed as cytotoxic antagonism was observed, indicating perhaps that the proliferating subpopulation of the tumor had been selected or induced for resistance properties that crossed from melphalan to thiotepa and carboplatin. Pharmacokinetic properties, that is, relatively slow distribution into the tumor of thiotepa and carboplatin, may have been a contributing factor in the magnitude of resistance observed. In contrast, when melphalan administration was followed 3 days later by treatment with high-dose cyclophosphamide, greater-than-additive cytotoxicity was seen. Subadditive cytotoxicity was observed with standard doses of cyclophosphamide, and with all but the highest dose of cyclophosphamide, no cytotoxic antagonism was seen with a 7-day interval.

Adriamycin is not a highly effective drug against the FSaIIC fibrosarcoma. Administration of a single dose of Adriamycin 3 days prior to that of melphalan, cyclophosphamide, or thiotepa did not alter the tumor-cell killing from that obtained with the alkylating agent alone.

Allowing an interval between drug treatments produces a biologically complex situation involving lysis and removal of dead cells as well as stasis or proliferation of surviving cells. Survival may be due to preexisting biochemical factors conferring resistance or induction of biochemical changes conferring resistance. With a single dose of drug, resistance development would be predicted to be transient and epigenetic; however, in the current study the 7-day interval between drug exposure was not sufficient to see reversion to sensitivity. Lengthening the interval between treatments may permit reversion to sensitivity; however, the concomitant increase in tumor-cell burden would be a great disadvantage in tumor treatment. The currently best solution may be to treat with the most effective agents early and to develop treatment sequences where overlapping mechanisms of resistance do not occur.

References

1. Alvarez Sotomayor E, Teicher BA, Schwartz GN, Holden SA, Meno K, Herman TS, Frei E (1992) Minocycline in combination with chemotherapy or radiation therapy in vitro and in vivo. Cancer Chemother Pharmacol 30: 377–384
2. Frei E III (1992) Pharmacologic strategies for high-dose chemotherapy. In: Armitage JO, Antman KH (eds) High-dose cancer therapy: pharmacology, hematopoietins, stem cells. Williams & Wilkins, Baltimore, pp 3–13
3. Frei E III, Antman KH (1993) Combination chemotherapy, dose, and schedule: section XV, principles of chemotherapy. In: Holland JF, Frei E III, Bast RC Jr, Kufe DW, Morton DL, Weichselbaum RR (eds) Cancer medicine Lea & Febiger, Philadelphia, pp 631–639
4. Frei E III, Teicher BA, Holden SA, Cathcart KNS, Wang Y (1988) Preclinical studies and clinical correlation of the effect of alkylating dose. Cancer Res 48: 6417–6423
5. Frei E III, Holden SA, Gonin R, Waxman DJ, Teicher BA (1994) Antitumor alkylating agents: in vitro cross-resistance and collateral sensitivity studies. Cancer Chemother Pharmacol (in press)
6. Holden SA, Teicher BA, Frei E III (1993) Stable and transient cross-resistance and collateral sensitivity among antitumor agents (AA). Proceedings 84th Meeting of the American Association for Cancer Research, Orlando, Florida, 34: 268
7. Rice L, Urano M, Suit HD (1980) The radiosensitivity of a murine fibrosarcoma as measured by three cell survival assays. Br J Cancer 41 [Suppl 4]: 240–245
8. Schabel FMJ, Trader MW, Laster WRJ, Wheeler GP, Witt MH (1978) Patterns of resistance and therapeutic synergism among alkylating agents. Antibiot Chemother 23: 200–215
9. Teicher BA (1992) Preclinical models for high-dose therapy. In: Armitage JO, Antman KH (eds) High-dose cancer therapy: pharmacology, hematopoietins, stem cells. Williams & Wilkins, Baltimore, pp 14–42
10. Teicher BA, Rose CM (1984) Perfluorochemical emulsion can increase tumor radiosensitivity. Science 223: 934–936
11. 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: 513–518
12. Teicher BA, Holden SA, Eder JP, Herman TS, Antman KH, Frei E III (1990) Preclinical studies relating to the use of thiotepa in the high-dose setting alone and in combination. Semin Oncol 17: 18–32