Cancer Chemother Pharmacol (1993) 32: 455–462

Cancer Chemotherapy and Pharmacology

Modulation of antitumor alkylating agents by novobiocin, topotecan, and lonidamine

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Received 14 January 1993/Accepted 28 April 1993

Abstract. Topoisomerase I and topoisomerase II allow a metabolically active cell to mobilize its supercoiled chromosomal DNA and undergo replication, transcription, recombination, and repair. Several topoisomerase inhibitors have recently been shown to be active in preclinical systems. Topotecan (SK&F 104864), a water-soluble camptothecin analog, is an inhibitor of topoisomerase I. Novobiocin is an inhibitor of toposiomerase II. Lonidamine depletes cellular adenosine 5′-triphosphate (ATP) and may impede energy-dependent DNA repair, MCF-7 human breast-cancer cells were treated in vitro with topotecan, novobiocin, and lonidamine alone, in paired combinations, and in combination with CDDP and melphalan. The three enzyme inhibitors alone and in combination did not increase tumor cell sensitivity to CDDP. However, the combinations of topotecan/novobiocin and lonidamine/novobiocin did enhance the cytotoxicity of melphalan. Mice bearing the FSaII fibrosarcoma were treated in vivo with topotecan, novobiocin, and lonidamine alone, in paired combinations, and in combination with CDDP, melphalan, BCNU, and cyclophosphamide. The combination of topotecan/novobiocin had the greatest impact on tumor cell sensitivity to each cytotoxic agent tested in both tumor cell-survival and tumor growth-delay assays. This sensitization was greatest at the highest concentrations of the cytotoxic agent tested. Combinations of topoisomerase I and topoisomerase II inhibitors may be useful as modulators of antitumor alkylating agents.

This work was supported by NIH grant P01-38493 and a grant from the Mathers Foundation

Abbreviations: CDDP, *cis*-diamminedichloroplatinum(II); thiotepa, *N,N′,N″*-triethylenethiophosphoramide; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; VM-26, 4′-demethylepipodophyllotoxin thenylidine-β-D-glucoside, teniposide; m-AMSA, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide; lonidamine, 1-[(2,4-dichlorophenyl)methyl]-1H-indazol-3-carboxglic acid

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Introduction

Chromosomal DNA is maintained in a supercoiled state via attachment to the nuclear matrix [55, 71]. During active processes such as DNA replication, transcription, recombination, and repair, the DNA topoisomerase I and II are required for unwinding of the supercoiled structure [8, 49, 71, 72, 74]. Topoisomerase I binds covalently to DNA, forming a single-strand break in the DNA through a phosphodiester bond. Topoisomerase I allows the passage of a single DNA strand; the strand break is then resealed using the energy preserved in the DNA phosphodiester bond [4, 5]. Topoisomerase II cleaves both strands of DNA and becomes covalently bound to a 5′-phosphoryl end of the DNA [72]. Topoisomerase II allows the passage of a double helix of DNA before resealing the strand breaks [26, 47, 73], a process that requires adenosine 5′-triphosphate (ATP) hydrolysis [54]. Experimental evidence thus far supports a direct role for topoisomerase I in transcription [28–30, 59, 60] and a direct role for topoisomerase II in DNA organization and replication [1, 2, 11, 19, 25, 35, 53, 75]. In the course of their activity, both topoisomerases cause unwinding of the double helix, rendering the DNA less compact and perhaps more susceptible to bifunctional antitumor alkylating agents or metallating agents such as the antitumor platinum complexes [13–15].

Camptothecin and several of its analogs have been shown to be inhibitors of topoisomerase I [18, 31, 32, 36, 37, 51, 52]. Topotecan (SK&F 104864), a water-soluble analog of camptothecin, has shown a broad spectrum of activity in preclinical systems and has recently completed phase I clinical trial [37, 51, 52, 58]. Novobiocin, an inhibitor of topoisomerase II, has myriad effects that occur at pharmacologically relevant concentrations and likely involve multiple mechanisms [3, 9, 12, 27, 33, 46, 50, 70]. Several clinical trials of novobiocin with antitumor alkylating agents have been carried out [16, 17].

Depletion of ATP can protect L1210 cells from the cytotoxic actions of topoisomerase II inhibitors such as VM-26 and m-AMSA but not from the cytotoxic actions of the topoisomerase I inhibitor camptothecin [45].

Lonidamine, a drug that affects the energy metabolism of cells, could be an important component of a combined modality regimen if repair of damage from cytotoxic treatment is an energy-dependent process [6, 7, 20–23, 61]. Lonidamine has inhibited the repair of potentially lethal damage caused by X-rays, methyl methane sulfonate, bleomycin, and hyperthermia in Chinese hamster HA-1 cells, and in murine tumor models it has potentiated the effects of radiation and the effects of hyperthermia [34, 41–43]. Lonidamine has also been shown to enhance the cytotoxicity of several antitumor alkylating agents in vitro and in vivo as well as Adriamycin in culture [57, 66–68, 76].

The current study was undertaken to extend our understanding of the modulation of antitumor alkylating agents in vitro and in vivo by the drugs topotecan, novobiocin, and lonidamine.

Materials and methods

Drugs. Topotecan (SK&F 104864) was a gift from Dr. Michael R. Mattern (SmithKline Beecham Pharmaceuticals, King of Prussia, Pa.). *cis*-Diamminedichloroplatinum(II) was a gift from Dr. Alfred Crosswell (Bristol-Myers-Squibb, Wallingford, Ct.). Lonidamine was obtained as a gift from DeSanctis Consultants (Montreal, Canada). Novobiocin, melphalan, thiotepa, and cyclophosphamide were purchased from Sigma Chemical Co. (St. Louis, Mo.). BCNU (carmustine) and ifosfamide were obtained from the Dana-Farber Cancer Institute Pharmacy.

Cell line. The MCF-7 human breast-adenocarcinoma cell line was developed by Dr. M. Rich of the Michigan Cancer Foundation. This line is estrogen-receptor-positive and retains certain characteristics of breast adenocarcinoma and has been widely used to study the response to antitumor alkylating agents [24, 63, 65, 69].

In vitro survival studies. MCF-7 cells in exponential growth were: (1) treated with various concentrations of each of the modulators novobiocin, topotecan, or lonidamine alone or in combination for 42 h, (2) treated with CDDP or melphalan at various concentrations for 1 h alone, or (3) treated with the modulators novobiocin (250 μM), topotecan (2.5 μM), or lonidamine (250 μM) for 42 h alone or in combination with exposure to various concentrations of CDDP or melphalan (1 h) during the 25th of modulator treatment. Following treatment, cells were washed three times with a 0.9% phosphate-buffered saline (PBS) solution and suspended by treatment with 0.25% trypsin/0.1% ethylenediaminetetraacetic acid (EDTA). The cells were plated in duplicate at three dilutions for colony formation. After 2 weeks, the colonies were visualized by staining with crystal violet and colonies of 50 cells or more were counted. The results were expressed as the surviving fraction of treated cells as compared with vehicle-treated control cells.

Tumor. The FSaII fibrosarcoma [56] adapted for growth in culture (FSaIIC) [64] was carried in male C3H/FeJ mice (Jackson Laboratory, Bar Harbor, Me.). For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of male C3H/FeJ mice 8–10 weeks of age.

Tumor excision assay. When the tumors had reached a volume of approximately 100 mm^3 (about 1 week after tumor cell implantation), five doses of novobiocin (50 mg/kg), five doses of lonidamine (50 mg/kg), or five doses of topotecan (50 mg/kg) were injected i.p. over 36 h alone or in combination with a single dose of antitumor alkylating agent given immediately after the fourth dose of the modulator(s). The doses of the alkylating agents (CDDP, 10, 20, or 30 mg/kg); melphalan, 5, 10, or 15 mg/kg; BCNU, 50, 100, or 200 mg/kg; and cyclophosphamide, 100, 300, or 500 mg/kg) were given i.p. to ensure that the modulators were present in the circulation at the time of alkylating agent treatment. Mice were killed 24 h after treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage. The tumors were excised under sterile conditions in a laminar flow hood and minced to a fine brei with two scalpels. Four tumors were pooled to make each treatment group. Approximately 300 mg of tumor brei was used to make each single-cell suspension. All reagents were sterilized with 0.22-μm Millipore membranes and were added aseptically to the tumor cells.

Each sample was washed in 20 ml of α-MEM (Grand Island Biological Co., Grand Island, N. Y.) in a 50-ml centrifuge tube, after which the liquid was gently decanted and discarded. The samples were resuspended in 450 units of collagenase/ml (Sigma) and 0.1 mg of DNase/ml (Sigma) and were incubated for 10 min at 37°C in a shaking water bath. The samples were centrifuged at 200 *g* and the supernatant was discarded. The samples were resuspended as described above and incubated for another 15 min at 37°C. The samples were then filtered through two layers of sterile gauze. The samples were washed twice and then resuspended in α-MEM supplemented with 10% fetal bovine serum (FBS; Sterile Systems, Logan, Utah). These single-cell suspensions were counted and plated at three different cell concentrations in duplicate for the colony-forming assay. After 1 week, the plates were stained with crystal violet and colonies of more than 50 cells were counted. The untreated tumor cell suspensions had a plating efficiency of 10%–16%. The results are expressed as the surviving fraction ± SE of cells from treated groups as compared with untreated controls.

Tumor growth-delay experiments. Treatment was initiated when the FSaII tumors had reached a volume of approximately 100 mm^3. Novobiocin (50 mg/kg), lonidamine (50 mg/kg), and/or topotecan (2.5 mg/kg) was injected i.p. once daily on days 6–13 after tumor implantation. CDDP (10 mg/kg) was given i.p. once on day 7. BCNU (15 mg/kg), cyclophosphamide (150 mg/kg), or ifosfamide (150 mg/kg) was injected i.p. once daily on days 7, 9, and 11. Thiotepa (5 mg/kg) was given i.p. once daily on days 7–11. The progress of each tumor was measured with calipers three times weekly until it reached a volume of 500 mm^3. Tumor growth delay was calculated as the number of days taken by each individual tumor to reach a volume of 500 mm^3 as compared with the untreated controls. Tumors in untreated control animals attained this volume in 12–14 days. Each treatment group consisted of five animals, and the experiment was repeated three times. Days of tumor growth delay are expressed as the mean ± SE for the treatment group as compared with the control.

Results

Neither novobiocin nor lonidamine was very cytotoxic toward exponentially growing MCF-7 human breast-carcinoma cells upon 42 h exposure to up to 500 μM of the drugs (Fig. 1). Topotecan, on the other hand, was quite cytotoxic toward MCF-7 cells upon 42 h exposure to the drug, such that 100 μM of topotecan killed about 3 logs of cells. MCF-7 cells were only moderately responsive to 1 h exposure to CDDP, with a concentration of about 155 μM resulting in a surviving fraction of 0.1 (Fig. 2). Each of the modulators increased the killing of MCF-7 cells by CDDP. Interestingly, the combination of the modulators in two cases was protective as compared with the cytotoxicity of the individual drugs. Thus, the surviving fraction for the combination of novobiocin (250 μM) with topotecan (2.5 μM) was 0.90 ± 0.07, whereas the surviving fractions for the individual agents were 0.88 ± 0.09 and 0.15 ± 0.07 for novobiocin (250 μM) and topotecan (2.5 μM), respectively. Similarly, the surviving fraction for the combination of topotecan (2.5 μM) and lonidamine (250 μM) was 1.00 ± 0.10, whereas the surviving fractions for the individual agents were 0.15 ± 0.07 and 0.78 ± 0.08 for topote-

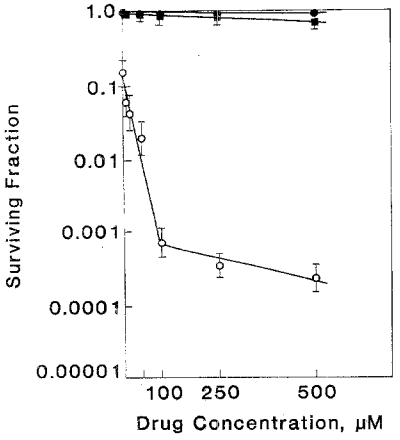

Fig. 1. Survival of exponentially growing MCF-7 human breast-carcinoma cells exposed for 24 h to various concentrations of novobiocin (●), topotecan (○), or lonidamine (■). *Points*, Means of 3 independent determinations; *bars*, SE

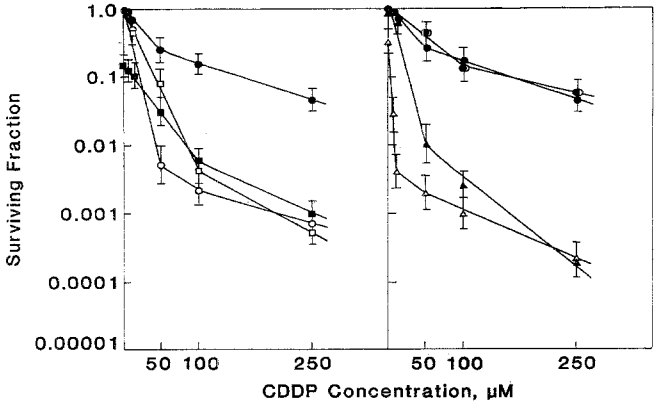

Fig. 2. Survival of exponentially growing MCF-7 human breast-carcinoma cells exposed to various concentrations of CDDP for 1 h alone (●) or during the 25th of a 42-h exposure to novobiocin (250 μ*M*, ○), topotecan (2.5 μ*M*, ■), lonidamine (250 μ*M*, ❑), novobiocin/topotecan (▲), novobiocin/lonidamine (△, or topotecan/lonidamine (○). *Points*, Means of 3 independent determinations: *bars*, SE

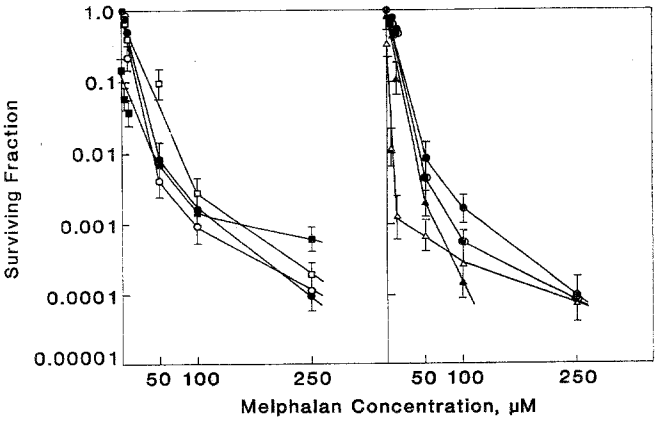

Fig. 3. Survival of exponentially growing MCF-7 human breast-carcinoma cells exposed to various concentrations of melphalan for 1 h alone (●) or during the 25th of a 42-h exposure to novobiocin (250 μ*M*, ○), topotecan (2.5 μ*M*, ■), lonidamine (250 μ*M*, ❑), novobiocin/topotecan (▲), novobiocin/lonidamine (△), or topotecan/lonidamine (○). *Points*, Means of 3 independent determinations; *bars*, SE

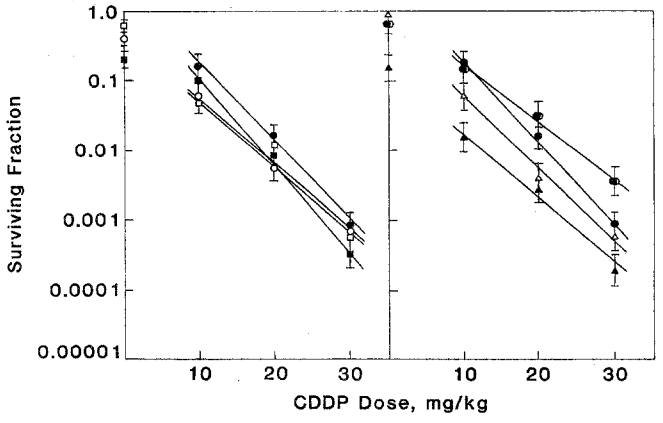

Fig. 4. Survival of FSaIIC cells from FSaIIC tumors treated with various doses of CDDP alone (●) or along with the third dose of a five-dose regimen of novobiocin (50 mg/kg, ○), topotecan (50 mg/kg, ■), lonidamine (50 mg/kg, ❑), novobiocin/topotecan (▲), novobiocin/lonidamine (△), or topotecan/lonidamine (●), *Points*, Means of 3 independent experiments; *bars*, SE

can (2.5 μ*M*) and lonidamine (250 μ*M*), respectively. The combination of novobiocin (250 μ*M*) with lonidamine (250 μ*M*) produced a surviving fraction of 0.34 ± 0.13, indicating that the combination of modulators resulted in greater than additive cytotoxicity as determined by the product of the surviving fractions. The modulator combinations of novobiocin/topotecan and novobiocin/lonidamine along with CDDP resulted, in general, in a 2- to 4-fold increase in the killing of MCF-7 cells over that obtained with CDDP and the single modulators. The modulator combination of topotecan/lonidamine completely negated the enhancing effects of topotecan and lonidamine as individual modulators and resulted in killing of MCF-7 cells that did not differ from that observed for CDDP alone.

Melphalan was a very effective cytotoxic agent toward MCF-7 cells, killing 1 log of cells at about 25 μ*M* and 3 logs of cells at about 125 μ*M* of the drug (Fig. 3). None of the single modulators was effective at increasing the killing of MCF-7 cells by melphalan. The modulator combinations of novobiocin/topotecan and novobiocin/lonidamine resulted in increased killing of MCF-7 cells by melphalan in the concentration range between 5 and 100 μ*M* of melphalan. The modulator combination of topotecan/lonidamine resulted in killing of MCF-7 cells that did not significantly differ from that achieved by melphalan alone.

To discern the effects of these modulators on the tumor cell killing by antitumor alkylating agents in vivo, animals bearing the FSaIIC fibrosarcoma were treated with five i.p. injections of the modulators given singly or in combination over 36 h. The antitumor alkylating agents were given in single i.p. injections alone or along with the third dose of the modulators. At 24 h after treatment with the antitumor alkylating agent, the tumors were excised and tumor cell survival was determined. CDDP produced log-linear killing of FSaIIC tumor cells with increasing doses of the drug (Fig. 4). Singly, the modulators produced relatively modest increases in the killing of FSaIIC cells by CDDP. The combination of novobiocin/topotecan produced a sur-

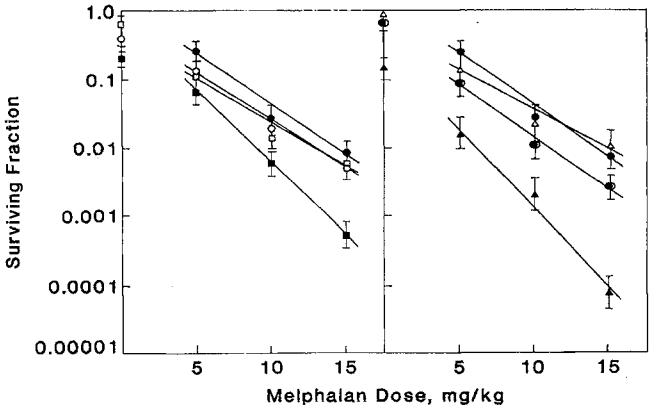

Fig. 5. Survival of FSaIIC cells from FSaIIC tumors treated with various doses of melphalan alone (●) or along with the third dose of a five-dose regimen of novobiocin (50 mg/kg, ○), topotecan (50 mg/kg, ■), lonidamine (50 mg/kg, ❑), novobiocin/topotecan (▲), novobiocin/lonidamine (△), or topotecan/lonidamine (●). *Points*, Means of 3 independent experiments; *bars*, SE

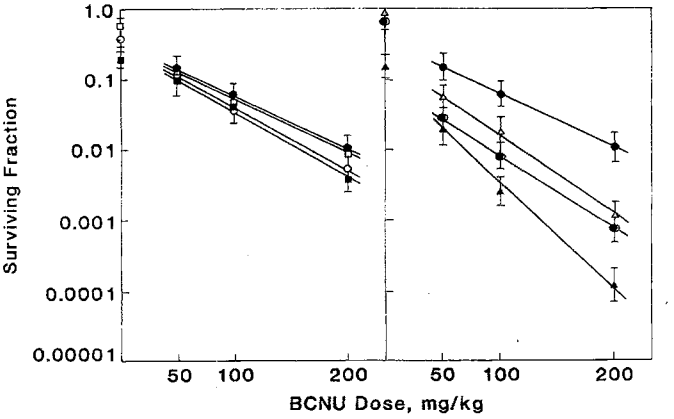

Fig. 7. Survival of FSaIIC cells from FSaIIC tumors treated with various doses of BCNU alone (●) or along with the third dose of a five-dose regimen of novobiocin (50 mg/kg, ○), topotecan (50 mg/kg, ■), lonidamine (50 mg/kg, ❑), novobiocin/topotecan (▲), novobiocin/lonidamine (△), or topotecan/lonidamine (●), *Points*, Means of 3 independent experiments; *bars*, SE

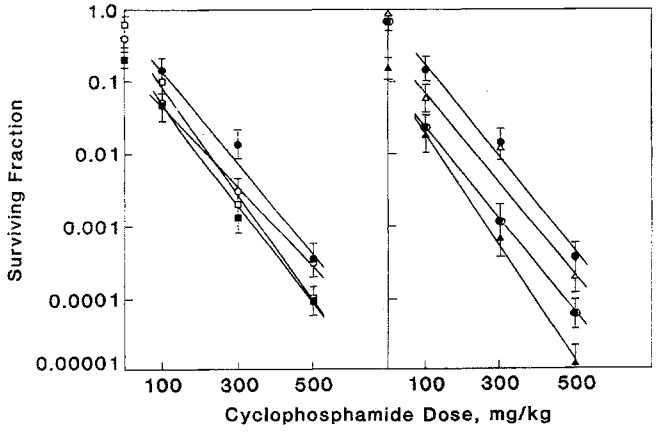

Fig. 6. Survival of FSaIIC cells from FSaIIC tumors treated with various doses of cyclophosphamide alone (●) or along with the third dose of a five-dose regimen of novobiocin (50 mg/kg, ○), topotecan (50 mg/kg, ■), lonidamine (50 mg/kg, ❑), novobiocin/topotecan (▲), novobiocin/lonidamine (△), or topotecan/lonidamine (●). *Points*, Means of 3 independent experiments; *bars*, SE

viving fraction of 0.15 ± 0.08, which represented less than additive killing by the individual modulators as determined by the product of the surviving fractions. Novobiocin/topotecan modulation resulted in about a 10-fold increase in FSaIIC tumor-cell killing at 10 mg/kg of CDDP, which decreased to about a 4-fold increase in tumor cell killing at the highest dose of CDDP tested. The combination of novobiocin/lonidamine produced about a 3-fold increase in the killing of FSaIIC tumor cells at the lower doses of CDDP but no significant increase in tumor cell killing at 30 mg/kg of CDDP. Topotecan/lonidamine did not alter the killing of FSaIIC tumor cells with lower doses of CDDP and was protective against tumor cell killing by high-dose CDDP.

Melphalan also produced a log-linear increase in FSaIIC tumor-cell killing with increasing doses of the drug (Fig. 5). Although novobiocin and lonidamine as single modulators did not significantly alter tumor cell killing by melphalan, topotecan was an effective modulator of melphalan, especially at higher doses of the drug. Novobiocin/topotecan modulation increased tumor cell killing by more than 1 log at the lower doses of melphalan and by 2 logs at the high dose of melphalan. Novobiocin/lonidamine did not alter FSaIIC tumor-cell killing from that obtained with melphalan alone. Topotecan/lonidamine increased FSaIIC tumor-cell killing by melphalan by about 3-times over the range of melphalan doses studied.

Cyclophosphamide given over the dose range of 100–500 mg/kg produced a log-linear increase in the killing of FSaIIC tumor cells with increasing doses of the drug (Fig. 6). The single modulators only modestly affected tumor cell killing by cyclophosphamide. Novobiocin/topotecan administration increased FSaIIC tumor-cell killing by about 1 log at the lower doses of cyclophosphamide, which increased to about 1.5 logs at the higher doses of cyclophosphamide. Novobiocin/lonidamine did not significantly alter the tumor cell killing from that produced by cyclophosphamide alone. Modulation by topotecan/lonidamine increased the tumor cell killing by cyclophosphamide by about 5-times.

Like the other antitumor alkylating agents, BCNU killed FSaIIC tumor cells in a log-linear manner (Fig. 7). The single modulators did not significantly alter the tumor cell killing from that obtained with BCNU alone. Modulation by novobiocin/topotecan resulted in about a 1-log increase in tumor cell killing by BCNU at the lower dose of BCNU, which increased to about a 2-log enhancement in tumor cell killing at the higher dose of the drug. Novobiocin/lonidamine modulation resulted in an enhancement in tumor cell killing by BCNU that increased from 3 to 10-times over the dose range of BCNU studied. Modulation by topotecan/lonidamine resulted in an enhancement in the killing of FSaIIC tumor cells that increased from 5 to 12-times over the dose range of BCNU studied.

Tumor growth-delay studies were conducted in the FSaIIC fibrosarcoma, with the single modulators and modulator combinations along with antitumor alkylating agents being given in full, standard-dose regimens (Tables 1, 2).

Table 1. Growth delay of the FSaIIC fibrosarcoma produced by various antitumor alkylating agents in combination with novobiocin, topotecan, or lonidamine as modulators[a]

Alkylating agent treatment	Tumor growth delay, days			
	Alone	+NOVO	+TOPO	+LOND
CDDP (10 mg/kg)	7.7±0.7	9.2±1.3	8.6±1.1	9.5±1.2
Cyclophosphamide (3×150 mg/kg)	9.3±1.2	14.4±1.8	21.3±2.3	17.4±2.4
Melphalan (10 mg/kg)	2.9±0.5	6.8±0.9	5.2±0.7	4.1±0.6
BCNU (3×15 mg/kg)	4.9±0.7	5.4±0.8	5.3±0.6	5.1±0.9
Thiotepa (5×5 mg/kg)	5.7±1.1	8.1±0.8	7.7±0.9	6.8±0.9
Ifosfamide (3×150 mg/kg)	6.2±1.0	9.0±0.7	10.1±1.3	8.3±1.1

[a] Tumor growth delay is the difference in the number of days required for treated tumors to reach a volume of 500 mm^3 as compared with untreated control tumors. The data are presented as the means of 15 animals ± SEM. The treatment schedules are given in Materials and methods. The tumor growth delays produced by the modulators alone were: (1) novobiocin (NOVO), 1.2±0.5 days; (2) topotecan (TOPO), 2.9±0.5 days; and (3) lonidamine (LOND), 2.0±0.4 days

Table 2. Growth delay of the FSaIIC fibrosarcoma produced by various antitumor alkylating agents and two modulator combinations[a]

Alkylating agent treatment	Tumor growth delay, days			
	Alone	+NOVO/TOPO	+NOVO/LOND	+TOPO/LOND
CDDP (10 mg/kg)	7.7±0.7	11.0±1.2	10.6±1.1	7.9±0.8
Cyclophosphamide (3×150 mg/kg)	9.3±1.2	48.5±2.7[b,*]	19.3±2.4[b,**]	39.2±2.7[b,*]
Melphalan (10 mg/kg)	2.9±0.5	10.2±1.4[b,**]	8.4±0.9[b,**]	9.5±1.1[b,**]
BCNU (3×15 mg/kg)	4.9±0.7	8.4±0.7	5.9±0.6	6.0±0.6
Thiotepa (5×5 mg/kg)	5.7±1.1	16.5±1.8[b]	8.7±1.1	9.8±1.3
Ifosfamide (3×150 mg/kg)	6.2±1.0	11.7±1.5	10.9±1.2	11.2±1.3

[a] Tumor growth delay is the difference in the number of days required for treated tumors to reach a volume of 500 mm^3 as compared with untreated control tumors. The data are presented as the means of 15 animals ± SEM. The treatment schedules are given in Materials and methods. The tumor growth delays produced by the modulators alone were: (1) novobiocin/topotecan (NOVO/TOPO), 4.0±0.6 days; (2) novobiocin/lonidamine (NOVO/LOND), 2.9±0.5 days; and topotecan/lonidamine, 2.4±0.5 days

[b] Significantly different from the corresponding drug-alone group: * $P<0.001$, ** $P<0.01$

The single modulators were variably effective in increasing the tumor growth delay produced by the various antitumor alkylating agents. Lonidamine was the most effective modulator of CDDP, increasing the tumor growth delay produced by CDDP by about 1.2 times. Treatment with the combination of topotecan/cyclophosphamide or topotecan/ifosfamide resulted in a tumor growth delay that was 2.3 and 1.6 times greater than that produced by cyclophosphamide or ifosfamide alone, respectively. The most effective modulator of melphalan and thiotepa was novobiocin, which increased the tumor growth delay produced by 2.3 and 1.4-times for melphalan and thiotepa, respectively. None of the single modulators significantly increased the tumor growth delay produced by BCNU.

Overall, the tumor growth delays resulting from treatment of animals bearing the FSaIIC fibrosarcoma with the antitumor alkylating agents and combined modulators were improved over those obtained with the antitumor alkylating agents and single modulators (Table 2). The most effective modulator combination was novobiocin/topotecan. Novobiocin/topotecan modulated CDDP such that the tumor growth delay produced by the combined treatment represented a 1.4-fold increase over that produced by treatment with CDDP. The tumor growth delay produced by cyclophosphamide with novobiocin/topotecan was 5.2 times greater than that resulting from treatment with cyclophosphamide alone. Melphalan was modulated by novobiocin/topotecan such that the tumor growth delay produced by the combination was 3.5 times greater than that produced by melphalan alone. The tumor growth delay produced by treatment with BCNU was increased 1.7 times by the addition of novobiocin/topotecan to treatment with the drug. Thiotepa was modulated by novobiocin/topotecan such that the tumor growth delay produced by the combination was 2.9 times greater than that produced by thiotepa alone. The tumor growth produced by treatment with ifosfamide was increased 1.9 times by the addition of novobiocin/topotecan to treatment with the drug.

Discussion

The cytotoxicity of combinations of CDDP with topoisomerase II inhibitors or topoisomerase I inhibitors has been studied in several different cell-culture systems and on different drug-exposure schedules [10, 13–15, 38, 39]. Defining additivity as the product of the surviving fractions for the drug treatments alone, Drewinko et al. [10] found that simultaneous exposure of cells to Adriamycin/CDDP or camptothecin/CDDP for 1 h resulted in greater than additive cytotoxicity. Katz et al. [39] found that continuous exposure (10 days) of human ovarian carcinoma cells to

CDDP and camptothecin or novobiocin resulted in additive cytotoxicity as determined by median-effect analysis. In the same study, the combination of etoposide and CDDP was determined to be less than additive in cytotoxicity. According to isobologram methodology, the combination of the camptothecin analog CPT-11 and CDDP resulted in greater than additive cytotoxicity toward leukemia cells upon a 3-day exposure to the drugs [38]. Novobiocin potentiated the cytotoxicity of a number of alkylating agents in vitro [13–15] and was synergistically cytotoxic in combination with CDDP and BCNU in Chinese hamster ovary cells. This increased cytotoxicity occurred in association with increased numbers of DNA interstrand cross-links being formed from monoadducts. Continuous novobiocin exposure was necessary to maximize the cross-link formation. Novobiocin has also been shown to increase the number of DNA interstrand cross-links formed by nitrogen mustard in a nitrogen mustard-resistant human Raji lymphoblastoid cell line that has elevated topoisomerase II levels [62]. Most recently, Lee et al. [46] found that novobiocin treatment protected Chinese hamster ovary cells from Adriamycin cytotoxicity but sensitized them to the toxic action of 4-hydroperoxycyclophosphamide.

Antagonism between topoisomerase I and topoisomerase II inhibitors as well as among topoisomerase II inhibitors in cell culture has been described in several reports [40, 46, 48, 70, 77]. In vivo, when the camptothecin analog CPT-11 was given 24 h prior to Adriamycin, the tumor growth delay of five human tumor xenografts was potentiated; however, simultaneous treatment with CPT-11 and Adriamycin was antagonistic, producing reduced tumor growth delay [44]. In these xenograft models, administration of CDDP 24 h after CPT-11 resulted in additive tumor growth delay. On the other hand, treatment of animals bearing the FSaII fibrosarcoma with novobiocin prior to, during, and after the administration of CDDP, BCNU, or cyclophosphamide produced an enhancement in tumor growth delay as compared with that observed for the antitumor alkylating agents alone [14].

In the current study, MCF-7 breast carcinoma cells were exposed to the topoisomerase II inhibitor novobiocin, the topoisomerase I inhibitor topotecan, or the ATP depletor lonidamine either alone or in two-drug combinations for 42 h. As observed by other investigators, the cytotoxicity of these modulators in combination was reduced as compared with that of the more cytotoxic single modulator. The enhancement in the cytotoxicity of CDDP (1 h exposure), however, was the same for the single and combined modulators, except for the combination of topotecan/lonidamine, where modulation was completely abolished. These enzyme inhibitors were very effective modulators of melphalan in cell culture, but the combinations of novobiocin/topotecan and novobiocin/lonidamine were more effective at lower concentrations of melphalan than were the single modulators or topotecan/lonidamine.

In vivo, additional factors such as drug penetration and metabolism, heterogeneity of cell-cycle distribution, and limitation of achievable drug levels may influence tumor cell killing by chemotherapeutic agents. As has been observed previously, CDDP, which is often highly "modulatable" in cell culture, is much less affected by drug combinations in vivo. The single modulators and topotecan/lonidamine did not improve tumor cell killing by CDDP; however, the combination of novobiocin/topotecan did lead to increased killing of FSaIIC tumor cells, as did modulation by novobiocin/lonidamine, albeit to a lesser degree than novobiocin/topotecan. The modulator combination of novobiocin/topotecan had a dose-modifying effect on tumor cell killing by melphalan, cyclophosphamide, and BCNU; that is, there was a steepening in the slope of the tumor-cell-killing curves for these antitumor alkylating agents with increasing doses of the drugs. This effect may indicate either an increase in the efficiency of DNA lesion formation (cross-link) by these drugs at higher doses, an increase in the efficiency of conversion of monoadducts to diadducts at higher alkylating agent doses, or a decrease in the efficiency of DNA lesion repair at higher doses of the alkylating agents. The combinations of lonidamine with novobiocin or topotecan were variably effective modulators, depending on the individual alkylating agent involved. Generally, modulator combinations were more effective than single modulators in enhancing the tumor cell killing by the antitumor alkylating agents.

In tumor growth-delay experiments with extended treatment regimens, the enhancement in the outcome of treatment with the antitumor alkylating agents by novobiocin/topotecan was most evident (the effect with CDDP remaining modest). As has been observed with other modulators, cyclophosphamide was the most positively affected antitumor alkylating agent, perhaps because cyclophosphamide is the single most effective alkylating agent against the FSaII fibrosarcoma.

Although combinations of topoisomerase II inhibitors or topoisomerase I and topoisomerase II inhibitors may be cytotoxically antagonistic in vitro and in vivo, this effect does not inhibit the ability of these agents to be effective modulators of antitumor alkylating agent tumor-cell killing.

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