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In vivo modulation of several anticancer agents by β-carotene

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Abstract. The ability of the collagenase inhibitor minocycline and of β-carotene to act as positive modulators of cytotoxic anticancer agents was assessed in vitro and in vivo. Cell-culture studies were conducted using the human SCC-25 squamous carcinoma cell line. Simultaneous exposure of the cells to minocycline and β-carotene or 13-*cis*-retinoic acid along with cisplatin (CDDP) resulted in a small decrease in the cytotoxicity of the CDDP. The addition of each of the modulator combinations for 1 h or 24 h to treatment with melphalan (L-PAM) or carmustine (BCNU) resulted in greater-than-additive cytotoxicity with each of four regimens. The modulator combinations of minocycline and β-carotene applied for 1 h or 24 h and the modulator combination of minocycline and 13-*cis*-retinoic acid produced greater-than-additive cytotoxicity at 50 μ*M* 4-hydroperoxycyclophosphamide (4-HC), whereas minocycline and 13-*cis*-retinoic acid applied for 1 h was antagonistic with 4-HC and the other modulator treatments at low concentrations of 4-HC resulted in subadditive cytotoxicity. The effect of treatment with β-carotene alone and in combination with several different anticancer agents was examined in two murine solid tumors, the FSaII fibrosarcoma and the SCC VII carcinoma. Administration of the modulators alone or in combination did not alter the growth of either tumor. Whereas increases in tumor growth delay occurred with the antitumor alkylating agents and β-carotene and with minocycline and β-carotene, a diminution in tumor growth delay was produced by 5-fluorouracil in the presence of these modulators. The modulator combination also resulted in increased tumor growth delay with Adriamycin and etoposide. Tumor-cell survival assay showed increased killing of FSaII tumor cells with the modulator combination and melphalan or cyclophosphamide as compared with the drugs alone. These results indicate that further investigation of this modulator strategy is warranted.

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Introduction

To maintain growth and allow invasion and metastases, solid tumors must develop a vasculature [9–15, 70, 72]. Initially tumors recruit a vascular supply from surrounding normal tissue; continued growth, however, requires induction of neovascularization (angiogenesis) [8–15, 53, 56, 72, 74]. The utility of an angiostatic agent in cancer therapy would be to inhibit the further growth of tumor masses, although retraction of some neovasculature may also be possible [11, 15]. It is also possible that damage to the existing tumor vasculature could make the tumor more susceptible to damage by cytotoxic cancer therapies [67].

The search for antiangiogenic substances has primarily led to the discovery of proteins and small molecules that inhibit various steps in the breakdown of the basement membrane [68, 69]. Many of these agents function by inhibiting enzymes that degrade the basement membrane. Therefore, in addition to being antiangiogenic, these treatments may function as antimetastatic agents by preventing invasion of tumor cells through the basement membrane [68, 69]. Among the recognized inhibitors of angiogenesis, retinoic acid (vitamin A) has been shown to inhibit neovascularization in the chick chorioallantoic membrane (CAM) assay [24, 29] and to alter the expression of collagen and lamimin in mouse F9 teratocarcinoma cells [71], and an analog of retinoic acid, etretinate, has demonstrated clinical activity in a well-vascularized tumor [51].

Among the enzymes that may be active in restructuring the extracellular matrix as tumors grow and develop vasculature, collagenase is an enzyme that degrades collagen IV and is secreted by both tumor cells and normal host cells. It has been recognized for some time that the tetracyclines can inhibit tissue collagenase activity, and tetracycline administration has been used in the treatment of several diseases where increased collagen degradation has been implicated [18–21, 63, 76]. Minocycline, a tetracycline, has been shown to inhibit neovascularization in rabbit corneas implanted with the VX2 carcinoma [63].

It has been demonstrated that the progression from premalignant dysplastic cells to malignant invasive tumor cells can be slowed, delayed, arrested, or even reversed in experimental animals by the administration of retinoids or carotenoids [25, 40–42, 44–48]. This effect has been seen in cells of epithelial or mesenchymal origin in which malignant changes have been induced chemically, virally, or via their transformation using radiation or growth factors. These findings have now been carried forward with some success into the clinic [25, 26].

However, as the toxicity of the retinoids has reduced their usefulness in the clinical treatment of cancers, recent studies have focused on the biologic and biochemical actions of the less toxic carotenoids, especially β-carotene [7, 30]. Over the past several years, work has focused on characterizing the prevention, inhibition, and regression of chemically induced experimental oral cancers of the hamster buccal pouch by β-carotene, other carotenoids, and vitamin E [40–42, 44–48, 52, 54, 56, 60, 61].

The current study was undertaken to compare the ability of the carotenoid β-carotene and the retinoid 13-*cis*-retinoic acid either alone or in combination with minocycline to modulate the activity of the antitumor agents in vitro or in vivo.

Materials and methods

Drugs. Minocycline, β-carotene, *cis*-diamminedichloroplatinum(II) (CDDP), melphalan (L-PAM), cyclophosphamide (CTX) and 5-fluorouracil (5-FU) were purchased from Sigma Chemical Co. (St. Louis, Mo.). 13-*cis*-Retinoic acid was a gift from Hoffman-LaRoche, Inc. (Nutley, N.J.). 4-Hydroperoxycyclophosphamide (4-HC) was a gift from Drs. P. Hilgard and J. Phol, Asta Pharma (Bielefeld, Germany). β-Carotene prepared for injection in a soy-based emulsion was obtained as a gift from Cardiospectrun, Inc. (Walpole, Mass.).

Cell line. SCC-25 human squamous head-and-neck carcinoma cells grow as monolayers in Dulbecco-Vogt modified Eagle's minimum essential medium (DMEM) supplemented with antibiotics and 5% fetal bovine serum (FBS) [16]. This cell line exhibits a plating efficiency of 10%–30% and a doubling time of 48–50 h in vitro [31]. For experiments, SCC-25 cells were grown in plastic culture flasks and harvested when they had reached the exponential growth phase [22].

Survival studies. SCC-25 cells in exponential growth were exposed either to various concentrations of minocycline, β-carotene, or 13-*cis*-retinoic acid alone or in combination for 1 h or 24 h or to various concentrations of CDDP, L-PAM, 4-HC, or BCNU for 1 h alone or in combination with minocycline (100 μ*M*) and β-carotene (50 μ*M*) or 13-*cis*-retinoic acid (50 μ*M*) for 1 h (during exposure to the alkylating agent) or for 24 h (with exposure to the alkylating agent being given during the 5th h).

Following treatment, cells were washed three times with phosphate-buffered 0.9% saline (PBS) and suspended by treatment with 0.25% trypsin, and known numbers of cells were then 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 were counted. The results were expressed as the surviving fraction of treated cells as compared with the vehicle-treated control.

Tumors. The FSaII fibrosarcoma [32] adapted for growth in culture (FSaIIC) [64] was carried in male C3H mice (Jackson Laboratory, Bar Harbor, Me.). The SCC VII tumor [2, 3] was carried in male C3H mice (Jackson Laboratory). 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 growth-delay experiments. By day 4 after tumor cell implantation the tumors had begun neovascularization [58, 67]. Animals bearing FSaII tumors were treated by i.p. injection with β-carotene (10 mg/kg) as a single dose, on alternate days for 3 doses or daily for 5 or 14 doses on days 7–11 or days 4–18, respectively. Minocycline (10 mg/kg) was given by i.p. injection daily for 14 days from day 4 through day 18. When the tumors had reached a volume of approximately 100 mm^3 (day 7 after tumor cell implantation), cytotoxic therapy was initiated. CDDP (10 mg/kg), L-PAM (10 mg/kg), and CTX (150 mg/kg) were injected i.p. on day 7. CTX (150 mg/kg) was also given on days 7, 9, and 11 following tumor implantation. Methotrexate (MTX, 0.8 mg/kg), 5-FU (40 mg/kg), and Adriamycin (1.75 mg/kg) were injected i.p. daily on days 7–11.

The progress of each tumor was measured thrice weekly until it had reached a volume of 500 mm^3. Tumor growth delay was calculated as the number of days required for each treated tumor to reach a volume of 500 mm^3 as compared with untreated control tumors. Each treatment group comprised five animals and the experiment was repeated three times. Tumor growth-delay data are shown as the mean value ± SE calculated for the treatment groups as compared with the control group.

Tumor excision assay. When the FSaIIC tumors had reached a volume of approximately 100 mm^3 (about 1 week after tumor cell implantation), the animals were treated with various doses of L-PAM, CTX, or CDDP or were given i.p. injections of β-carotene (5×5 mg/kg) or minocycline (5×5 mg/kg) over 24 h, with the cytotoxic drugs being given with the third modulator dose. 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 [65]. The plating efficiency of untreated tumor-cell suspensions ranged from 10% to 16%. The results were expressed as the surviving fraction (±SE) of cells from treated groups as compared with untreated controls [65].

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 a granulocyte/macrophage colony-forming unit (CFU-GM) assay was carried out as previously described [65]. Colonies of at least 50 cells were scored on an Acculite colony counter (Fisher Scientific, Springfield, N.J.). The results of three experiments, in which each group was measured in duplicate at three cell concentrations, were averaged. The results were expressed as the surviving fraction of treated groups as compared with untreated controls.

Isobologram analysis. Isobologram analysis was carried out as previously described [66]. Dose-response curves for each agent alone were generated first. Combinations producing an effect that was within the envelope boundaries of modes I and II generated with a computer program were considered to be additive; those displaced to the left were greater than additive (i.e., supra-additive), whereas those displaced to the right were less than additive (i.e., subadditive) [66].

Results

Minocycline was not very cytotoxic toward exponentially growing human SCC-25 squamous carcinoma cells following 1 h or 24 h exposure to concentrations of up to 500 μ*M* of the drug (Fig. 1). Both β-carotene and 13-*cis*-retinoic acid were cytotoxic to SCC-25 cells. β-Carotene was more cytotoxic than 13-*cis*-retinoic acid for the same concentration and exposure time. In all, 90% of the SCC-25 cells were killed by 530 μ*M* of 13-*cis*-retinoic acid after a

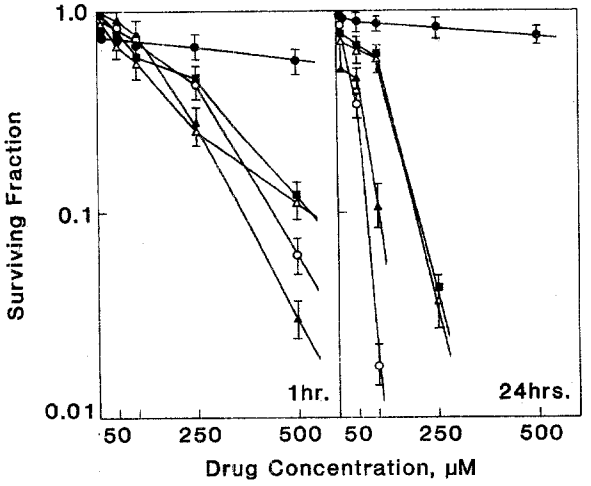

Fig. 1. Survival of exponentially growing human SCC-25 squamous carcinoma cells exposed for 1 h or 24 h to various concentrations of minocycline (●), β-carotene (○), 13-*cis*-retinoic acid (■), minocycline (100 μ*M*) + β-carotene (▲), and minocycline (100 μ*M*) + 13-*cis*-retinoic acid (Δ). *Points*, Mean values for 3 independent experiments; *bars*, SEM

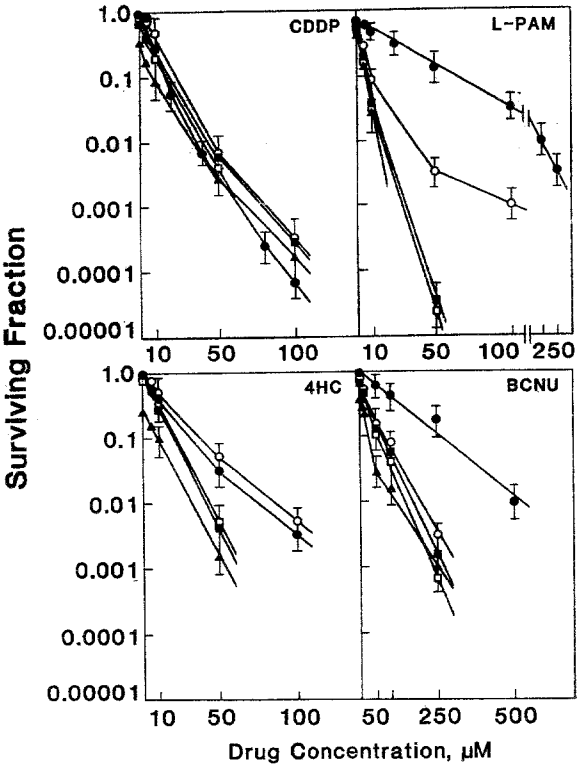

Fig. 2. Survival of exponentially growing human SCC-25 squamous carcinoma cells exposed for 1 h to various concentrations of CDDP, L-PAM, 4-HC, or BCNU alone (●), along with minocycline (100 μ*M*) and 13-*cis*-retinoic acid (50 μ*M*; ○), along with minocycline (100 μ*M*) and β-carotene (50 μ*M*; ■), or along with minocycline (100 μ*M*) and 13-*cis*-retinoic acid (50 μ*M*; ❑) or minocycline (100 μ*M*) and β-carotene (50 μ*M*; ▲) for 24 h, with antitumor alkylating-agent treatment being given during the 5th h of modulator exposure. *Points*, Mean values for 3 independent experiments; *bars*, SEM

1-h exposure and by 200 μ*M* of 13-*cis*-retinoic acid after a 24-h exposure. The addition of 100 μ*M* of minocycline to treatment with 13-*cis*-retinoic acid did not alter the cytotoxicity of the 13-*cis*-retinoic acid. Of the SCC-25 cells, 90% were killed by 440 μ*M* of β-carotene upon 1 h exposure and by 105 μ*M* of β-carotene after 24 h exposure. The addition of 100 μ*M* of minocycline to treatment with β-carotene led to increased cytotoxicity such that 360 and 70 μ*M* of β-carotene produced 90% cell killing after exposure periods of 1 h and 24 h, respectively.

Survival curves generated for exponentially growing human SCC-25 squamous carcinoma cells exposed for 1 h to CDDP, L-PAM, 4-HC, or carmustine (BCNU) are shown in Fig. 2. Simultaneous exposure of the cells to minocycline (100 μ*M*) and β-carotene (50 μ*M*) or 13-*cis*-retinoic acid (50 μ*M*) along with CDDP resulted in a small decrease in the cytotoxicity of the CDDP. Exposure of the cells for 24 h to minocycline (100 μ*M*) and 13-*cis*-retinoic acid (50 μ*M*), with CDDP treatment occurring during the 5th h of modulator exposure, did not alter the cytotoxicity of CDDP at the level of 90% cell killing, whereas the same treatment with minocycline (100 μ*M*) and β-carotene (50 μ*M*) resulted in additive cytotoxicity of the two treatments.

The addition of the modulator combinations for 1 h or 24 h to treatment with L-PAM resulted in greater-than-additive cytotoxicity with each of four regimens (Fig. 2). A level of 90% cell killing was achieved with 63 μ*M* of L-PAM alone and with 7–10 μ*M* of L-PAM in combination with the modulators. The modulator combinations of minocycline (100 μ*M*) and β-carotene (50 μ*M*) applied for 1 h or 24 h and the modulator combination of minocycline (100 μ*M*) and 13-*cis*-retinoic acid (50 μ*M*) produced greater-than-additive cytotoxicity at 50 μ*M* L-PAM. In contrast, minocycline (100 μ*M*) and 13-*cis*-retinoic acid (50 μ*M*) applied for 1 h was antagonistic with 4-HC, and the other modulator treatments at low concentrations of 4-HC resulted in subadditive cytotoxicity. The addition of any of the modulator combinations for 1 h or 24 h to treatment of the SCC-25 cells with BCNU resulted in greater-than-additive cytotoxicity toward the cells.

The effect of treatment with β-carotene alone and in combination with several different anticancer agents was examined in two murine solid tumors, the FSaII fibrosarcoma and the SCC VII carcinoma (Table 1).

In the FSaII fibrosarcoma the β-carotene emulsion was applied as a single dose on day 7 immediately prior to the cytotoxic drug, on alternate days (days 7, 9, and 11) for three injections, or daily for 5 days. β-Carotene administration produced minimal, if any tumor growth delay on any of the three schedules. In contrast, when a single dose of β-carotene preceded administration of L-PAM, there was a 1.4-fold increase in tumor growth delay as compared with treatment with L-PAM alone; however, when L-PAM treatment was preceded by a dose of β-carotene and the β-carotene administration was continued for 4 more days, a 4.3-fold increase in tumor growth delay occurred. In the SCC VII tumor the same treatment regimen resulted in a 2.1-fold increase in tumor growth delay as compared with treatment with L-PAM alone. Preceding administration of a single dose of CDDP with a single dose of β-carotene produced a 1.3-fold increase in tumor growth delay as compared with treatment with CDDP alone; however, when

Table 1. Growth delay of the FSaII fibrosarcoma and the SCC VII carcinoma produced by treatment with β-carotene and antitumor agents

Treatment group	Tumor growth delay (days)[a]			
	Drug alone	β-Carotene[b]		
		X1	X3	X5
FSaII fibrosarcoma:				
–	–	0.3±0.3	0.8±0.3	1.2±0.4
L-PAM (10 mg/kg)	2.7±0.4	3.7±0.5	–	11.7±0.9
CDDP (10 mg/kg)	7.5±0.6	9.5±1.1	–	–
CTX (150 mg/kg)	4.3±0.5	8.0±0.8	–	–
CTX (3×150 mg/kg)	7.8±0.7	–	13.5±1.5	19.0±1.8
BCNU (3×15 mg/kg)	2.5±0.4	–	5.3±0.5	–
5-FU (5×40 mg/kg)	7.6±0.7	–	–	2.3±0.4
MTX (5×0.8 mg/kg)	2.2±0.4	–	–	2.7±0.5
SCC VII carcinoma:				
–				0.3±0.3
L-PAM (10 mg/kg)	2.7±0.3			5.8±0.6
CDDP (10 mg/kg)	6.7±0.6			6.8±0.7
CTX (3×150 mg/kg)	13.5±1.5			18.6±1.7
BCNU (3×15 mg/kg)	3.2±0.4			3.4±0.5
5-FU (5×40 mg/kg)	2.4±0.3			2.2±0.3
Adria (5×1.75 mg/kg)	1.6±0.3			1.7±0.3

Adria, Adriamycin

[a] Tumor growth delay is defined as the number of days required for treated tumors to reach 500 mm^3 as compared with untreated control tumors. Each data point represents results from 15 animals

[b] β-Carotene (10 mg/kg) was given by i.p. injection of a soy-based emulsion. Each anticancer drug was given at a standard dose and schedule for that drug. All drugs were given by i.p. injection on the following schedules: as a single dose, on alternate days for three doses, or daily for 5 days. Within each subgroup of treatments, β-carotene was given on the schedule shown with each agent

Table 2. Growth delay of the FSaII fibrosarcoma produced by long-term treatment with β-carotene and minocycline and antitumor agents

Treatment group	Tumor growth delay (day)[a]	
	Drug alone	β-Carotene/ minocycline[b]
–	–	0.3±0.3
L-PAM (10 mg/kg)	2.7±0.4	9.7±0.9
CDDP (10 mg/kg)	7.5±0.6	9.3±1.1
CTX (3×150 mg/kg)	7.8±0.7	10.0±1.2
Ifosfamide (3×150 mg/kg)	6.3±0.7	8.8±1.2
5-FU (5×40 mg/kg)	7.6±0.7	3.2±0.4
Etoposide (5×15 mg/kg)	4.9±0.7	16.8±1.7
Adriamycin (5×1.75 mg/kg)	8.9±1.0	28.9±2.7

[a] Tumor growth delay is defined as the number of days required for treated tumors to reach 500 mm^3 as compared with untreated control tumors. Each data point represents results from 5 animals

[b] β-Carotene (10 mg/kg) was given by i.p. injection daily on days 4–18 after tumor implantation of a soy-based emulsion. Minocycline (10 mg/kg) was injected i.p. daily on days 4–18. Each anticancer drug was given on days 7–11 at a standard dose and schedule for that drug. All drugs were given by i.p. injection on the following schedules: as a single dose, on alternate days for three doses, or daily for 5 days. Within each subgroup of treatments, β-carotene was given on the schedule shown with each agent

β-carotene was given prior to and for 4 days after CDDP in the SCC VII, there was no increase in tumor growth delay as compared with administration of CDDP alone. β-Carotene given prior to CTX or daily over the course of CTX administration resulted in a 1.7- to 2.4-fold increase in FSaII tumor growth delay, whereas daily administration of β-carotene along with a course of CTX resulted in a 1.4-fold increase in tumor growth delay as compared with treatment with CTX alone. Administration of β-carotene along with BCNU resulted in a 2.1-fold increase in the tumor growth delay of the FSaII fibrosarcoma, but no significant growth delay for the SCC VII tumor in response to BCNU was observed. Preceding each dose of 5-FU with β-carotene resulted in a marked (3.3-fold) decrease in the response of the FSaII fibrosarcoma to the drug. β-Carotene administration also failed to affect the response of the SCC VII tumor to Adriamycin.

The combination of the modulators β-carotene (10 mg/kg) and minocycline (10 mg/kg) administered daily over the tumor growth period from day 4 after tumor cell implantation to day 18 following tumor cell implantation did not alter the growth pattern of the tumor as compared with untreated controls (Table 2). This long-term modulator treatment regimen resulted in equal or less enhancement of the response of the FSaII fibrosarcoma to the antitumor alkylating agents than was obtained with the 5-day course of β-carotene alone. The decreased response to 5-FU was

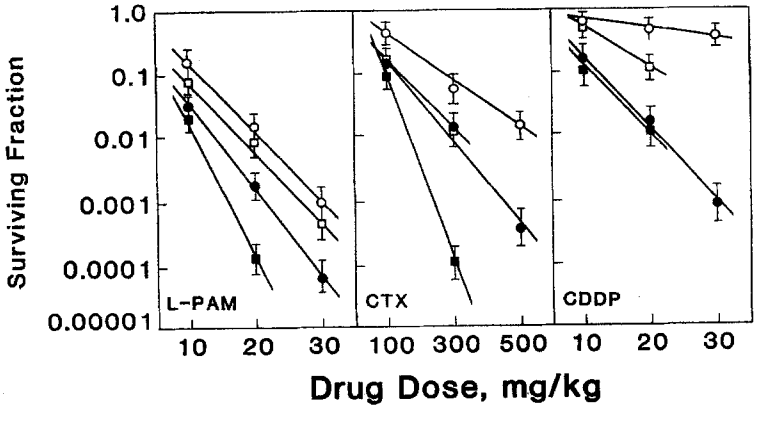

Fig. 3. Survival of FSaIIC cells (●, ■) and bone marrow CFU-GM (○, ❑) from animals bearing FSaIIC tumors that were treated with various doses of L-PAM, CTX, or CDDP alone (●, ○) or combined with β-carotene (5×5 mg/kg) and minocycline (5×5 mg/kg; ■, ❑) given over 24 h. The cytotoxic drugs were given as a single i.p. injection immediately after the third modulator dose. *Points*, Mean values for 3 independent experiments; *bars*, SEM

the same with the modulator combination as with the five daily doses of β-carotene alone.

Significantly, the modulator combination of β-carotene and minocycline was very effective at increasing the response of the FSaII fibrosarcoma to etoposide and Adriamycin. There was a 3.4-fold increase in the tumor growth delay produced by Adriamycin given along with the modulator combination as compared with the drugs given alone.

FSaII tumor-cell and bone-marrow CFU-GM survival studies showed that administration of the modulator combination β-carotene (5 mg/kg) and minocycline (5 mg/kg) for five doses over 24 h, with the antitumor alkylating agent being given with the third modulator dose, did not alter the killing of FSaII tumor cells by CDDP (Fig. 3). It appeared, however, that treatment with the modulators along with CDDP (20 mg/kg) resulted in increased cytotoxicity to bone marrow CFU-GM. In contrast, the modulator combination produced a dose-modifying effect, a change in the slope of the tumor cell-killing curve for both L-PAM and CTX in the FSaII fibrosarcoma. For example, a dose of 20 mg/kg of L-PAM produced about 1 log of increased tumor cell killing with the modulators. A dose of 300 mg/kg of CTX produced about 2 logs of increased tumor cell killing with the modulators as compared with the drugs alone. The increased tumor cell killing was also associated with the cytotoxicity of bone marrow CFU-GM. The modulator combination and L-PAM produced about a 2-fold increase in cytotoxicity as compared with L-PAM alone. The increase in the killing of bone marrow CFU-GM by CTX plus the modulator combination was 2.7- to 5-fold that obtained with CTX alone.

Discussion

Through the clinical observation of high-risk populations and through preclinical modeling it has become evident that malignant disease develops over time from premalignant lesions [17, 23]. There is also evidence to support the notion that carcinogenesis or progress toward malignancy can be reversed in the preinvasive stages. Extensive clinical work has been carried out especially in head and neck disease using treatment with retinoids and/or β-carotene to prevent the progress of premalignant conditions to head and neck cancers. There has been a great of success in these clinical trials, and work is continuing to establish the best agents for these relatively long-term therapies [25, 26]. The mechanism(s) by which retinoids act at the cellular level involve regulation of gene expression [25, 29, 72].

Retinoids and carotenoids have been shown to regulate the differentiation of various cells [57]. In addition, retinoids and carotenoids can regulate the expression of protooncogene and oncogenes such as *c-fos*, *c-jun*, *c-myc*, and mutant p53 [5, 6, 27, 37, 50, 59]. Although 13-*cis*-retinoic acid and β-carotene have been implicated in the initiation of apoptosis, their relationship to programmed cell death requires further analysis [1].

Retinoids can also alter the metabolic state of cells containing enzymes such as alcohol dehydrogenase, phospho(enol)pyruvate, pyruvate carboxykinase, and transglutaminase [57]. Retinoids initiate cell changes through the activation of a super family of nuclear receptors that elaborate a series of transcription factors. The other initiators of this superfamily of receptors include vitamin D, thyroid hormone, and glucocorticoids [33]. A recent study has also indicated that retinoids can interact with growth factors such as transforming growth factor β_1(TGF-β_1) [62]

Carotenoids such as β-carotene act as prooxidant oxygen-labile molecules, reducing the activity of superoxide dismutase and glutathione-S-transferase (GST) as well as the level of non-protein sulfhydryls [34]. In addition, levels of stress proteins (hsp) 70 and 90 are increased [21, 43, 49]. β-Carotene also reduces the expression of epidermal growth factor and transforming growth factor, and levels can be increased by exposure to β-carotene [43]. In contrast, an increased expression of TGF-β1 and tumor necrosis factor-α has been noted in carcinoma and premalignant sites following the administration of β-carotene [36, 38, 55].

Among the steps in the progress toward malignancy is the conversion from a preangiogenic to an angiogenic phenotype [8–10, 13, 28]. The generation of blood vessels allows growth and invasion [8–10, 13, 28]. Therefore, it is likely that among the critical genes controlled by the retinoids or carotenoids to induce differentiation and suppress proliferation in premalignant conditions are genes involved in signaling the initiation of angiogenesis [35, 48].

The role of retinoids and carotenoids in chemoprevention may be regarded as well established [4, 17, 23, 25, 26, 39, 75]; however, the possible role of these agents as modulators of cytotoxic therapies in the treatment of established malignant disease is only now being explored. Recently, Lippman et al. [26] showed that treatment of patients with locally advanced squamous-cell carcinoma of the cervix with the combination of 13-*cis*-retinoic acid and interferon-α, a known antiangiogenic agent [28, 73], produced a very high response rate in this disease, although "resistance" seemed to develop relatively easily to this therapy since all of the responders relapsed while on the therapy. Cell-culture studies indicated that 13-*cis*-retinoic acid and β-carotene were similarly cytotoxic; therefore, we initiated in vivo studies with β-carotene, which is known to be much less toxic to normal tissues in animals. We have begun integrating antiangiogenic modulation with cytotoxic therapies in preclinical solid tumors [58, 67]. The availability of an injectable emulsion preparation of β-carotene allowed systemic administration of this modulator. Not unexpectedly, for an agent that suppresses proliferation, administration of β-carotene interacted negatively in vivo with the cell-cycle-specific anticancer drug 5-FU.

The actions of retinoids and carotenoids on proliferating cells appear to be, in general, to suppress the proliferative phenotype, thereby reversing premalignant conditions and preventing progress toward malignancy. These agents may also find a role in conjunction with cytotoxic therapies in the treatment of established malignant disease.

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