

# Differential Response of Human Ovarian Cancer Cells to Induction of Apoptosis by Vitamin E Succinate and Vitamin E Analogue, $\alpha$ -TEA

Kristen Anderson,<sup>1</sup> Marla Simmons-Menchaca,<sup>1</sup> Karla A. Lawson,<sup>2</sup> Jeffrey Atkinson,<sup>3</sup> Bob G. Sanders,<sup>1</sup> and Kimberly Kline<sup>2</sup>

<sup>1</sup>School of Biological Sciences/C0900 and <sup>2</sup>Division of Nutrition/A2703, University of Texas at Austin, Austin, Texas, and <sup>3</sup>Department of Chemistry, Brock University, St. Catharines, Ontario, Canada

## ABSTRACT

A vitamin E derivative, vitamin E succinate (VES; RRR- $\alpha$ -tocopheryl succinate), and a vitamin E analogue, 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy acetic acid ( $\alpha$ -TEA), induce human breast, prostate, colon, lung, cervical, and endometrial tumor cells in culture to undergo apoptosis but not normal human mammary epithelial cells, immortalized, nontumorigenic breast cells, or normal human prostate epithelial cells. Human ovarian and cervical cancer cell lines are exceptions, with  $\alpha$ -TEA exhibiting greater proapoptotic effects. Although both VES and  $\alpha$ -TEA can induce A2780 and subline A2780/cp70 ovarian cancer cells to undergo DNA synthesis arrest within 24 h of treatment, only  $\alpha$ -TEA is an effective inducer of apoptosis. VES or  $\alpha$ -TEA treatment of cp70 cells with 5, 10, or 20  $\mu$ g/ml for 3 days induced 5, 6, and 19% versus 9, 36, and 71% apoptosis, respectively. Colony formation data provide additional evidence that cp70 cells are more sensitive to growth inhibition by  $\alpha$ -TEA than VES. Differences in stability of the ester-linked succinate moiety of VES versus the ether-linked acetic acid moiety of  $\alpha$ -TEA were demonstrated by high-performance liquid chromatography analyses that showed  $\alpha$ -TEA to remain intact, whereas VES was hydrolyzed to the free phenol, RRR- $\alpha$ -tocopherol. Pretreatment of cp70 cells with *bis*-(*p*-nitrophenyl) phosphate, an esterase inhibitor, before VES treatment, resulted in increased levels of intact VES and apoptosis. Taken together, these data show  $\alpha$ -TEA to be a potent and stable proapoptotic agent for human ovarian tumor cells and suggest that endogenous ovarian esterases can hydrolyze the succinate moiety of VES, yielding RRR- $\alpha$ -tocopherol, an ineffective apoptotic-inducing agent.

## INTRODUCTION

Studies in our lab have focused on the antitumor properties of vitamin E compounds. Two compounds in particular, RRR- $\alpha$ -tocopheryl succinate (vitamin E succinate; VES), a vitamin E derivative, and a novel vitamin E analogue, 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy acetic acid, referred to as  $\alpha$ -TEA, differ in structure from natural vitamin E (RRR- $\alpha$ -tocopherol) by the addition of a succinic acid group attached at the C6 position of the chroman head structure by an ester linkage (VES) or an acetic acid moiety attached at the C6 position of the chroman head by an ether linkage ( $\alpha$ -TEA; Fig. 1; Refs. 1, 2). VES has been shown to be a potent growth inhibitor of a wide variety of cancer cell lines *in vitro*, as well as an effective tumor growth inhibitor *in vivo* (1, 3–14). Recently,  $\alpha$ -TEA has been shown to reduce tumor burden and lung metastasis in a syngeneic mouse mammary cancer model (2).

Human tumor cells in culture undergo DNA synthesis arrest, dif-

ferentiation, and apoptosis in response to treatment with VES (reviewed by Ref. 1; Refs. 15–21). Studies on the mechanisms of actions of VES using human breast cancer cells show that MDA-MB-435 cells are nonresponsive to the growth inhibitory properties of transforming growth factor  $\beta$  and Fas/Fas ligand; however, after treatment with VES, responsiveness to both transforming growth factor  $\beta$  and Fas is restored (reviewed by Ref. 1; Refs. 22, 23). Prolonged activation of the c-Jun-NH<sub>2</sub>-terminal kinase is a key event in VES-induced apoptosis (reviewed by Ref. 1; Ref. 17), and recent studies have shown that c-Jun-NH<sub>2</sub>-terminal kinase plays a role in Bax translocation to the mitochondria, which results in increased mitochondrial membrane permeability with release of cytochrome *c*, activation of caspases 9 and 3, and apoptosis (19). Recent studies have demonstrated that  $\alpha$ -TEA-induced apoptosis is similar to VES-induced apoptosis in that transforming growth factor  $\beta$ -, Fas-, and c-Jun-NH<sub>2</sub>-terminal kinase signaling pathways are necessary, at least in part for  $\alpha$ -TEA-induced apoptosis (24). Ovarian cancer is the most lethal of the female gynecologic cancers, and there is a great need for novel chemotherapeutic agents to treat this cancer (25). Thus, it was of interest to see if VES or the novel vitamin E analogue  $\alpha$ -TEA might be effective agents.

Here, we report that: (a)  $\alpha$ -TEA is as effective as VES in inducing apoptosis in human breast, prostate, colon, lung, and endometrial tumor cells in culture and is superior to VES as an inducer of apoptosis in human ovarian and cervical tumor cells; (b) although VES is less effective as an inducer of apoptosis in ovarian cp70 cells, VES and  $\alpha$ -TEA are equally effective in inducing DNA synthesis arrest, demonstrating a unique difference between how these two compounds signal DNA synthesis arrest and apoptosis; and (c) ovarian cells contain esterases that can hydrolyze the ester linked succinate moiety from VES yielding RRR- $\alpha$ -tocopherol, an ineffective apoptotic-inducing agent. Taken together, these studies demonstrate that  $\alpha$ -TEA is a stable compound and a potent inducer of apoptosis in a wide variety of human cancer cells in culture, including human ovarian and cervical cells. In comparison, VES is an effective proapoptotic agent for a wide variety of human cancer cells but is less effective than  $\alpha$ -TEA in inducing human ovarian and cervical cancer cells to undergo apoptosis.

## MATERIALS AND METHODS

**Cell Lines and Culture Conditions.** Sources of cell lines used in these studies are as follows. MDA-MB-435 [provided by Dr. Janet E. Price, Department of Cell Biology, University of Texas M. D. Anderson Cancer Center (Houston, TX)], MDA-MB-231 (American Type Culture Collection, Manassas, VA), and MCF-7 [provided by Dr. Suzanne Fuqua, Baylor College of Medicine (Houston, TX)] are human breast cancer cell lines; MCF-10A (American Type Culture Collection) is an immortalized, nontumorigenic human breast cell line, and human mammary epithelial cells (Cooperative Human Tissue Network, Birmingham, AL) were primary cultures of human mammary cells derived from normal mammoplasty specimens. All of the breast-derived cell lines and primary cells were cultured as described previously (23). LNCaP, PC-3, and DU-145 are human prostate carcinoma cell lines [provided by Dr. Claudio Conti, University of Texas M. D. Anderson-Science Park Research Division (Smithville, TX)]. Normal human prostate epithelial cells were pur-

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**Note:** K. Lawson is currently at the Cancer Prevention Fellowship Program, Division of Cancer Prevention, National Cancer Institute, Bethesda, MD.

**Requests for reprints:** Kimberly Kline, Division of Nutrition/A2703, University of Texas at Austin, Austin, TX 78712-1097. Phone: (512) 471-8911; Fax: (512) 232-7040; E-mail: k.kline@mail.utexas.edu.

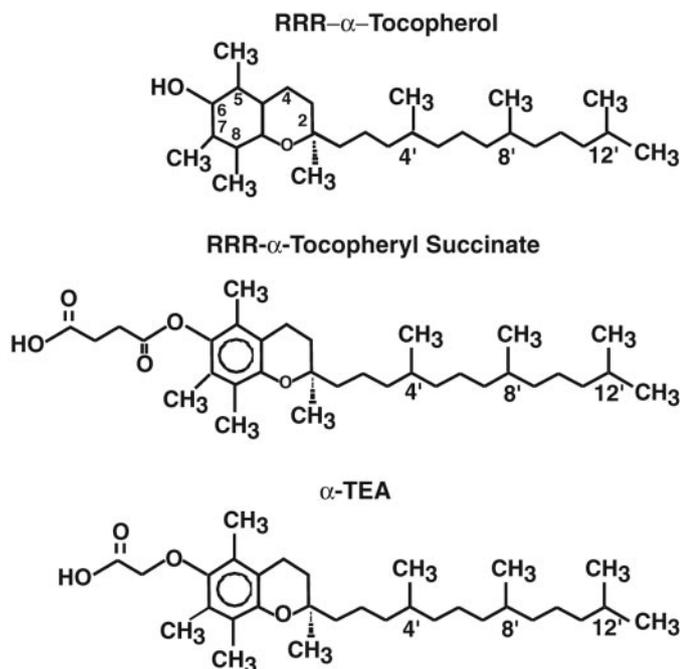


Fig. 1. Structure of the parent compound RRR- $\alpha$ -tocopherol; vitamin E-derivative RRR- $\alpha$ -tocopheryl succinate (VES), possessing an ester-linked succinate moiety attached to 6C of the chroman head of RRR- $\alpha$ -tocopherol; and vitamin E analogue [2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yl]oxy] acetic acid ( $\alpha$ -TEA), possessing an ether-linked acetic acid moiety attached to the 6C of the chroman head of RRR- $\alpha$ -tocopherol.

chased from Clonetics (San Diego, CA). All of the prostate-derived cell lines and primary cells were cultured as described previously (26). The human colon HT-29 and lung A-549 were purchased from the American Type Culture Collection and cultured according to American Type Culture Collection's instructions. The human cervical ME-180 and endometrial RL-95-2 were a kind gift from Dr. Michael J. Birrer (NIH, Rockville, MD). The human cervical SiHA and CaSki cell lines were obtained from Dr. Rebecca Richards-Kortum (University of Texas at Austin). The human ovarian 2008 and cisplatin-resistant subclone, 2008-C13, were obtained from Dr. Stephen Howell (University of California, San Diego, CA). The ovarian carcinoma cell lines OVCA-429, OVCA-433, OVCA 432, HEY, and SK-OV-3 were obtained from Dr. Gordon Mills (University of Texas M. D. Anderson Cancer Center).

The human ovarian A2780 cisplatin-sensitive parental cancer cell line was provided by Dr. J. Rebecca Liu (University of Michigan Medical School, Ann Arbor, MI), and the cisplatin-resistant A2780/cp70 subclone, referred to as cp70, was provided by Dr. Michael J. Birrer. The cp70 subclone was created through intermittent exposure of A2780 cells to increasing concentrations of cisplatin (up to 70 mM) *in vitro* (27). A2780 and cp70 cells were grown as monolayers on plastic (Corning Plastic Ware, Corning, NY) and maintained in RPMI 1640 (Invitrogen-Life Technologies, Inc., Carlsbad, CA), supplemented with 10% fetal bovine serum (Gemini Bio-Products, Woodland, CA), 100 IU/ml penicillin, 100  $\mu$ g/ml streptomycin, and 2 mM glutamine (Sigma Chemical Co., St. Louis, MO). Cultures were routinely examined and shown to be free of *Mycoplasma* contamination.

**$\alpha$ -TEA and VES Treatments.** For experiments, human mammary epithelial cells were cultured in mammary epithelial growth media, and prostate epithelial cells were cultured in prostate epithelial growth medium, both of which are defined media without serum purchased from Clonetics (San Diego, CA), PC-3 cells in 1% fetal bovine serum, MDA-MB-435, and LNCaP cells in 2% fetal bovine serum and all other cell types were cultured in 5% fetal bovine serum. Cp70 cells were plated at a density of  $1 \times 10^5$  cells/ml in T-75 flasks (20 ml) for high-performance liquid chromatography (HPLC) analyses, T-25 flasks (10 ml) for Western analyses, 12-well plates (1 ml) for apoptosis, or 96-well plates (100  $\mu$ l) for DNA synthesis arrest and allowed to adhere overnight before treatment initiation. Treatments were conducted at various concentrations of VES (Sigma Chemical Co.) or  $\alpha$ -TEA (synthesized by one of the authors, Dr. Jeffrey Atkinson, and described in detail; Ref. 2) in a final

concentration of 0.1% ethanol. Vehicle treatments consisted of an equivalent amount of sodium succinate for the highest amount of VES used in the experiment in a final concentration of 0.1% ethanol.

**Esterase Inhibitor Analyses.** Bis-(*p*-nitrophenyl)phosphate (BNPP), an inhibitor of nonspecific esterases that prevents de-esterification of VES (28), was used to determine whether esterase activity could be responsible for the inability of VES to induce apoptosis in the cp70 cells. Cp70 cells at  $1.5 \times 10^5$  cells/ml in 20 ml/T-75 flask were pretreated with 12.5 or 25 mM BNPP for 30 min before the addition of 5, 10, or 20  $\mu$ g/ml VES. Cells were cultured for 1, 2, and 3 days, harvested, and analyzed for apoptosis using procedures described below. Cell lysates were analyzed for intact VES *versus* free RRR- $\alpha$ -tocopherol by HPLC analyses as described below.

**DNA Synthesis Assay.** Detection of DNA synthesis arrest of cp70 cells in response to treatments with VES or  $\alpha$ -TEA was assessed by measuring [ $^3$ H]thymidine incorporation as described previously (29). Briefly, cp70 cells at  $1 \times 10^5$  cells/ml in 96-well plates (100  $\mu$ l/well) were cultured with 5, 10, and 20  $\mu$ g/ml VES or  $\alpha$ -TEA for 24 h. During the last 4 h of incubation, cultures were pulsed with 0.5  $\mu$ Ci [ $^3$ H]thymidine. Cells were harvested and [ $^3$ H]thymidine uptake measured using a Beckman LS5000TD liquid scintillation counter.

**Apoptotic Analyses of 4',6-Diamidino-2-Phenylindole Dihydrochloride-Stained Cells.** Apoptosis was assessed based on nuclear morphology using the fluorescent DNA dye 4',6-diamidino-2-phenylindole, as described previously (23). Cells in which the nucleus contained clearly condensed chromatin or cells exhibiting fragmented nuclei were scored as apoptotic. Apoptotic data are reported as the percentage of apoptosis obtained by determining the number of apoptotic cells *versus* the total number of cells. For each sample, a minimum of three slides involving >500 cells/slide were scored. Apoptotic data are presented as mean  $\pm$  SD for three independent experiments.

**Poly(ADP-Ribose) Polymerase (PARP) Cleavage Detection by Western Blot.** Cleavage of PARP by caspase 3 serves as an early indicator of apoptosis (30). Cp70 cells were plated at a density of  $1.5 \times 10^5$  cells/ml in T-25 flasks (10 ml/flask) and allowed to adhere overnight. Next, the media were removed and replaced with experimental media containing treatments of VES,  $\alpha$ -TEA, vehicle, or VES + BNPP (25 mM), and cells were allowed to incubate for 2 days. Cells were collected by scraping to combine floating and adherent cells and then pelleted by centrifugation at  $350 \times g$ . Whole cell lysates were prepared and analyzed by Western blotting performed as previously described with the exception that proteins were separated using SDS-PAGE on a 7.5% gel (23). Proteins were visualized with immune-enhanced chemiluminescence analyses using 1  $\mu$ g of primary antibody [PARP (H-250), Santa Cruz Biotechnology, Santa Cruz, CA] and peroxidase-conjugated goat antirabbit immunoglobulin as the secondary antibody (Jackson Immunoresearch Laboratory, West Grove, PA) at a 1:1000 dilution.

**Colony Assay.** Clonogenic assays were performed on exponentially growing cells using published procedures (31). Briefly, cp70 cells, a total of 1000 to  $1 \times 10^6$ , for low to high doses of treatment, respectively, was seeded in  $35 \times 10$ -mm Petri dishes in 2 ml of normal growth media and allowed to adhere overnight. Growth media were removed and replaced with 2 ml of treatment media containing VES (2.5, 5, and 10  $\mu$ g/ml),  $\alpha$ -TEA (2.5, 5, and 10  $\mu$ g/ml), or untreated. After 9 days of treatment, media were carefully removed, and the cells were stained with 0.1% methylene blue for 30 min. Cells were washed once with PBS, and colonies of >50 cells were counted as survivors. The surviving fraction was calculated for each treatment based on the plating efficiency of the untreated sample, using the formula: surviving fraction = (number of colonies counted at a given level of VES or  $\alpha$ -TEA/number of colonies plated)  $\times$  (control number of cells plated/control number of colonies counted). Each experiment was repeated at least three times.

**Lipid Extraction of Samples.** Lipid extraction was performed on whole cell lysates of cp70 samples to determine the amount of cellular uptake of VES and  $\alpha$ -TEA. Cells were extracted according to the method of Tirmenstein *et al.* (32), with slight modifications. Fifty  $\mu$ l of internal standard, RRR- $\delta$ -tocopherol (1 mg/ml in ethanol; Sigma Chemical Co.) were added to each sample in disposable 5-ml centrifuge tubes (Sarstedt catalogue no. 58.536; Sarstedt, Inc., Newton, NC) with five to seven Kimble solid glass beads (4 mm). According to the method of Knight *et al.* (33), cells were resuspended in 0.1 M SDS and homogenized on a mini-beadbeater (model no. 3110B; Crescent Wig-I-bug -Dentsply, Elgin, IL) device for 30 s. Two ml of ethanol were then added to each vial and mixed by vortexing. Homogenates were extracted twice with

hexane by adding 1 ml of hexane, followed by shaking in the Wig-I-bug for 30 s, centrifugation for 5 min at 1000 × g, and organic layer removed. Hexane extracts of each sample were combined and evaporated under nitrogen and then resuspended in 200 μl of methanol.

**HPLC Analyses.** RRR-α-tocopherol, VES, and α-TEA levels were measured by an internal standard method using reverse-phase HPLC with fluorometric detection as described by Timenstein *et al.* (32). Forty μl of each sample in methanol were injected into a Waters 717 HPLC equipped with an autosampler. The mobile phase consisted of 96% methanol (HPLC grade; Aldrich, Milwaukee, WI), 4% water, and 0.001% glacial acetic acid. Samples were separated on a Waters spherisorb ODS-2 5u (250 × 4.6-mm) column (Alltech, Deerfield, IL). Excitation and emission wavelengths of 210 and 300 nm, respectively, were used for all determinations. Quantitation of the separated compounds was performed based on the internal standard method using RRR-δ-tocopherol as the internal standard and Millennium-32 chromatography manager software for data analyses (Waters Corp., Milford, MA). α-TEA, VES, and RRR-α-tocopherol peaks were collected, and mass spectrometry with elemental analysis was performed to verify their chemical composition.

**RESULTS**

**Ability of VES and α-TEA to Inhibit Growth of Human Cancer Cells but not Normal or Immortalized Cells.** A comparison of the ability of VES and α-TEA to induce human breast, prostate, colon, lung, ovarian, cervical, and endometrial cancer cells to undergo apoptosis is shown in Table 1. These data show that VES and α-TEA behave in a similar manner in the induction of apoptosis with all tumor cells tested, with the exception of the ovarian and cervical cancer cells. Furthermore, normal human mammary epithelial cells, normal prostate epithelial cells, and immortalized, nontumorigenic breast cells (MCF-10A) were refractive to the apoptotic-inducing

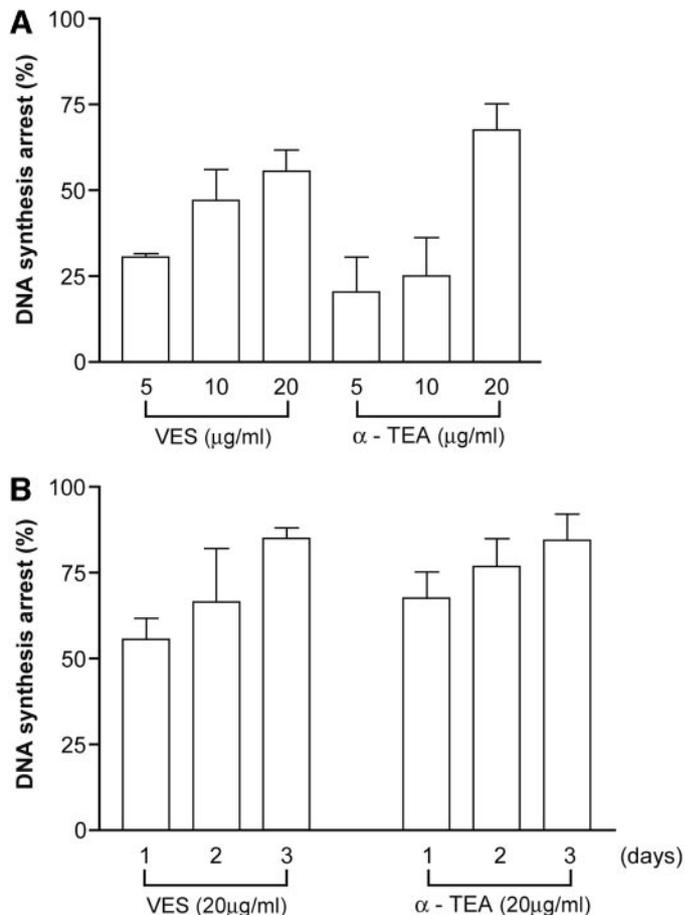


Fig. 2. Vitamin E succinate (VES) and α-TEA induce cp70 cells to undergo DNA synthesis arrest. Cp70 cells at 1 × 10<sup>5</sup> cells/ml in 96-well plates (100 μl/well) were cultured with 5, 10, and 20 μg/ml VES or α-TEA for 1 day (A) or 20 μg/ml of each compound for 1, 2, and 3 days (B). Four h before harvesting, cells were pulsed with 0.5 mCi [<sup>3</sup>H]thymidine. Cells were harvested and uptake of [<sup>3</sup>H]thymidine determined. Data are depicted as mean ± SD for three independent experiments.

properties of both VES and α-TEA. The superior ability of α-TEA, in comparison to VES, to induce apoptosis was initially found in the cp70 and A2780 ovarian cells, with a suggestion that perhaps α-TEA was also more effective in inducing apoptosis in the ME-180 cervical cells. To determine whether this finding was more generalizable, 7 additional ovarian cell lines and 2 additional cervical cell lines were analyzed (Table 1). Data showed α-TEA to be more effective than VES in inducing apoptosis for both ovarian and cervical cell lines (Table 1). The difference in ability of VES and α-TEA to induce apoptosis in cp70 cells formed the basis for additional investigation.

**VES and α-TEA Treatment of Cp70 Cells Induce DNA Synthesis Arrest.** Cp70 cells treated with VES or α-TEA undergo DNA synthesis arrest (Fig. 2, A and B). Treatment of cp70 cells with 5, 10, and 20 μg/ml VES or α-TEA for 1 day induced 31, 47, and 56% and 20, 25, and 68% DNA synthesis arrest, respectively (Fig. 2A). Treatment of cp70 cells with 20 μg/ml VES or α-TEA for 1, 2, and 3 days induced 56, 67, and 85% and 68, 77, and 85% DNA synthesis arrest, respectively (Fig. 2B).

**α-TEA Is Effective in Inducing Cp70 Cells to Undergo Apoptosis; VES Is Less Effective.** Treatment of cp70 cells with VES for 2 and 3 days at 5, 10, and 20 μg/ml gave low levels of apoptosis; 3, 17, and 19% for day 2, and 5, 6, and 20% apoptosis for day 3, respectively (Fig. 3, A and B), whereas treatment of cp70 cells in a similar manner with α-TEA gave 3, 26, and 73% apoptosis for day 2 and 9, 36, and 71% apoptosis for day 3 (Fig. 3, A and B).

Table 1 Comparison of the apoptotic inducing properties of VES<sup>a</sup> and α-TEA, using several different human cancer cell lines<sup>b</sup>

Human cancer cell lines	Apoptosis Induction <sup>c</sup>	
	VES	α-TEA
<b>Breast</b>		
MDA-MB-435	5–10	5–10
MDA-MB-231	5–10	5–10
MCF-7	10–20	5–10
<b>Cervical</b>		
ME-180	10–20	1–5
SiHA	10–20	1–5
CaSki	15–20	1–5
<b>Ovarian</b>		
Cp-70 <sup>d</sup>	>40	5–10
A2780	>40	5–10
2008	>40	5–10
2008-C13	10–15	5–10
Hey	>40	5–10
OVCA-429	>40	15–20
OVCA-433	>40	15–20
OVCA-432	>40	10–15
SK-OV-3	15–20	10–15
<b>Endometrial</b>		
RL-95-2	10–20	10–20
<b>Prostate</b>		
LnCaP	5–10	5–10
PC-3	5–10	5–10
DU-145	5–10	5–10
<b>Colon</b>		
HT-29	5–10	5–10
<b>Lung</b>		
A-549	20–30	20–30

<sup>a</sup> VES, vitamin E succinate; α-TEA, 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl)chroman-6-yloxy acetic acid.

<sup>b</sup> Human cancer cells were cultured with 5, 10, 20, and 40 μg/ml VES or α-TEA for 2 days and scored for apoptosis, using 4',6-diamidino-2-phenylindole staining of nuclei.

<sup>c</sup> Apoptotic data are depicted as range of compound from experiment to experiment that induces 50% (EC<sub>50</sub>) of cells to undergo apoptosis. Background levels of apoptosis in vehicle-treated cells and untreated cells ranged between 3 and 5%.

<sup>d</sup> Treatment of ovarian cancer cells for 2 days at the highest concentration, 40 μg/ml, failed to produce an EC<sub>50</sub>.

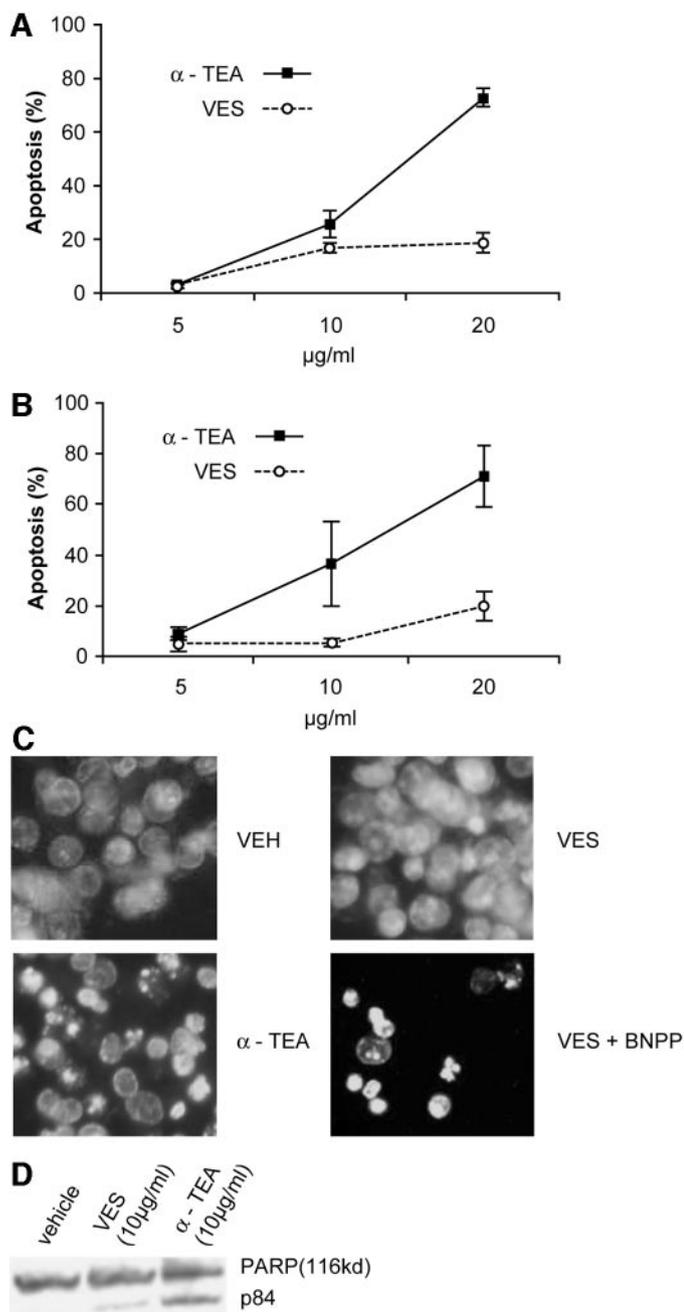


Fig. 3. α-TEA is an effective apoptotic-inducing agent, vitamin E succinate (VES) is less effective. Cp70 cells were cultured at  $1 \times 10^5$  cells/ml in 12-well plates with 5, 10, and 20 µg/ml VES or α-TEA for 2 (A) and 3 (B) days. Floating and adherent cells were harvested, washed, and stained with 4',6-diamidino-2-phenylindole dihydrochloride. Apoptosis was determined by counting the number of cells exhibiting condensed and fragmented nuclear morphology ( $>500$  cells/slide) and reported as percent apoptosis. Data for A and B are depicted as the mean  $\pm$  SD for three independent experiments. Photomicrographs of 4',6-diamidino-2-phenylindole dihydrochloride-stained cp70 cells cultured with vehicle (VEH), 20 µg/ml VES or α-TEA, and pretreated with 25 mM esterase inhibitor bis-(p-nitrophenyl) phosphate (BNPP) for 30 min followed by treatment with 20 µg/ml VES are depicted in C. Cell lysates from cp70 cells cultured with 10 µg/ml VES or α-TEA for 2 days were analyzed by Western immunoblotting for cleavage of caspase 3 substrate poly(ADP-ribose) polymerase (PARP). PARP cleavage fragment has a  $M_r$  of  $\sim 84,000$  (D).

Nuclei of VEH- and VES (20 µg/ml)-treated cp70 cells exhibited uniform dull staining typical of normal intact cells (Fig. 3C, top panels). When cp70 cells were cultured with 20 µg/ml VES + 25 mM esterase inhibitor BNPP, VES induced cp70 cells to undergo apoptosis as depicted by condensed and fragmented nuclei (Fig. 2C, bottom

right panel). Nuclei from α-TEA-treated cells showed nuclear condensation and fragmentation (Fig. 3C, bottom left panel). The cp70 parental cells, A2780, also exhibited resistance to VES-induced apoptosis (data not shown).

Additional evidence of apoptosis induced by α-TEA but not with VES was obtained by Western immunoblotting analyses of the caspase 3 substrate PARP (Fig. 3D). Cell lysates from cells treated with 10 µg/ml VES for 1 day showed low levels of PARP cleavage fragment p84, whereas cell lysates from cells treated with 10 µg/ml α-TEA showed the expected  $M_r$  84,000 PARP cleavage fragment, providing additional support that α-TEA is an effective inducer of apoptosis in cp70 cells, whereas VES is less effective (Fig. 3D).

**α-TEA Is More Effective Than VES in Inhibiting *In Vitro* Colony Formation by Cp70 Cells.** In the colony formation study, the plating efficiency for cp70 cells cultured in media only for 9 days was 46%. The surviving fraction of cp70 cells after treatment for 9 days with 2.5, 5, and 10 µg/ml VES was  $1.20 \pm 0.14$ ,  $0.46 \pm 0.34$ , and  $0.06 \pm 0.02$ , respectively, whereas the surviving fraction of cells treated for the same time period with 2.5 and 5 µg/ml α-TEA was  $0.62 \pm 0.23$  and  $0.03 \pm 0.014$ , respectively (Fig. 4). No surviving colonies were formed in plates treated with 10 µg/ml α-TEA at any cell concentration tested, including the highest cell number ( $1 \times 10^6$ ). The colony formation data provide additional evidence that cp70 cells are more sensitive to growth inhibition by α-TEA than VES.

**HPLC Analyses of Levels and Integrity of VES and α-TEA in Cp70 Cell Lysates.** Levels of VES and α-TEA in cp70 cells increased over 3–6 h after treatment, reaching maximum levels at 12 h following treatment (data not shown). HPLC data depicted in Fig. 5, A and B, are from cells treated for 12 h with α-TEA or VES, respectively. HPLC analyses of cell lysates from α-TEA-treated cp70 cells showed only the α-TEA peak eluting in 11 min, indicating that α-TEA remained structurally intact (Fig. 5A). HPLC analyses of cell lysates from VES-treated cells showed a VES peak eluting at 12 min, as well as a second peak that eluted from the column at 14 min. Mass spectrometry with elemental analysis confirmed the second peak to be RRR-α-tocopherol, providing direct evidence that the ester-linked succinate moiety of VES had been cleaved (Fig. 5B).

Studies designed to determine the effect of RRR-α-tocopherol on VES or α-TEA uptake and apoptosis involved treating cp70 cells with VES or α-TEA singly or in combination with RRR-α-tocopherol, followed by analyses of cell lysates by HPLC or cell populations for

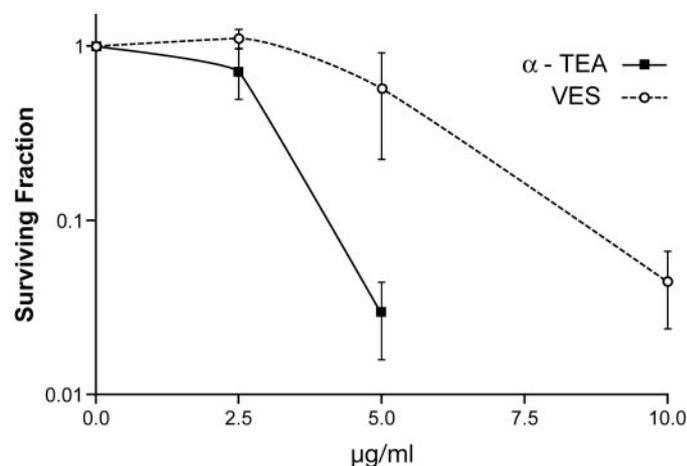


Fig. 4. Clonogenic assay performed on exponentially growing cells cultured with 2.5, 5, or 10 µg/ml vitamin E succinate (VES) or α-TEA. After 9 days of culture, media were removed, cells stained with 0.1% methylene-blue, and colonies counted. Surviving fraction for each treatment was calculated based on the plating efficiency of the untreated sample, as described in "Materials and Methods." Data are depicted as the mean  $\pm$  SD of three independent experiments.

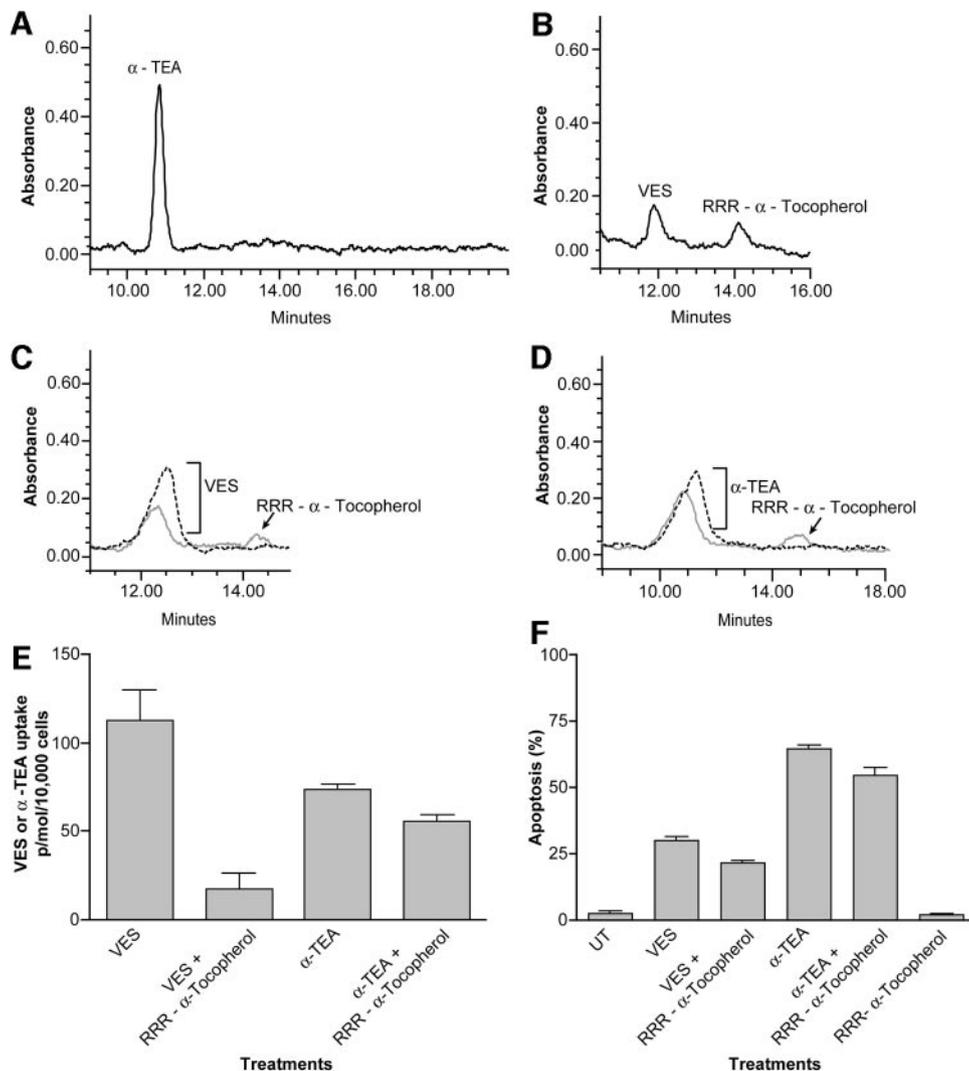


Fig. 5. High-performance liquid chromatography analyses were conducted, as described in "Materials and Methods," on cell lysates from  $1 \times 10^5$  cp70 cells/ml cultured (10-ml volume) in 25-ml flasks with 20  $\mu\text{g/ml}$  α-TEA (A), 20  $\mu\text{g/ml}$  vitamin E succinate (VES; B), or 20  $\mu\text{g/ml}$  VES with (light solid line) or without (darker dotted line) 100  $\mu\text{g/ml}$  RRR-α-tocopherol (C) or 20  $\mu\text{g/ml}$  α-TEA with (light solid line) or without (darker dotted line) 100  $\mu\text{g/ml}$  RRR-α-tocopherol (D) for 12 h. VES and α-TEA levels in cells treated with single or combination treatments with RRR-α-tocopherol are depicted in pmol/10,000 cells as the mean  $\pm$  SD of three independent experiments (E). Cp70 cells were untreated (UT) or treated for 3 days with 20  $\mu\text{g/ml}$  VES or α-TEA with and without treatment with 100  $\mu\text{g/ml}$  RRR-α-tocopherol or 100  $\mu\text{g/ml}$  RRR-α-tocopherol alone. Cells were collected and apoptosis determined by 4',6-diamidino-2-phenylindole dihydrochloride staining as described previously (F). Apoptotic data are depicted as mean  $\pm$  SD of three independent experiments. A, B, and C data are representative of multiple experiments.

number of apoptotic cells. HPLC data showed that both VES or α-TEA were taken up by the cells when administered in combination with RRR-α-tocopherol, but VES and α-TEA levels in cotreated cells were lower than singly treated cells (Fig. 5, C and D, respectively). Quantitation of levels of VES or α-TEA uptake/10,000 cells shows that single versus cotreated cells exhibited mean  $\pm$  SD of  $113 \pm 34.6$  versus  $18 \pm 12.06$  pmol VES and  $74 \pm 4.24$  versus  $55 \pm 4.95$  pmol α-TEA (Fig. 5E). Analyses of the effects of various treatments on induction of apoptosis in cp70 cells showed that RRR-α-tocopherol alone did not induce the cells to undergo apoptosis and that combination treatments of RRR-α-tocopherol with either VES or α-TEA resulted in decreased percentage of apoptotic cells of 28 and 13% in comparison to VES and α-TEA treatments alone (Fig. 5F).

**VES Induces Cp70 Cells to Undergo Apoptosis When Cells Are Pretreated with Cellular Esterase Inhibitor BNPP Followed by Treatment with VES.** Pretreatment of cp70 cells with 12.5 or 25 mM of the cellular esterase inhibitor BNPP for 30 min did not cause the cells to undergo apoptosis (Fig. 6A). Pretreatment of cells with 25 mM BNPP for 30 min, followed by treatment with VES at 5, 10, and 20  $\mu\text{g/ml}$  for 2 days induced 12, 19, and 37% apoptosis in comparison to cells treated with VES alone which induced 3, 8, and 19%, respectively (Fig. 6A). Pretreatment of cells with BNPP had no effect on α-TEA induced apoptosis (data not shown).

Additional data showing that treatment with 25 mM BNPP for 30 min enhances VES-induced apoptosis come from Western immuno-

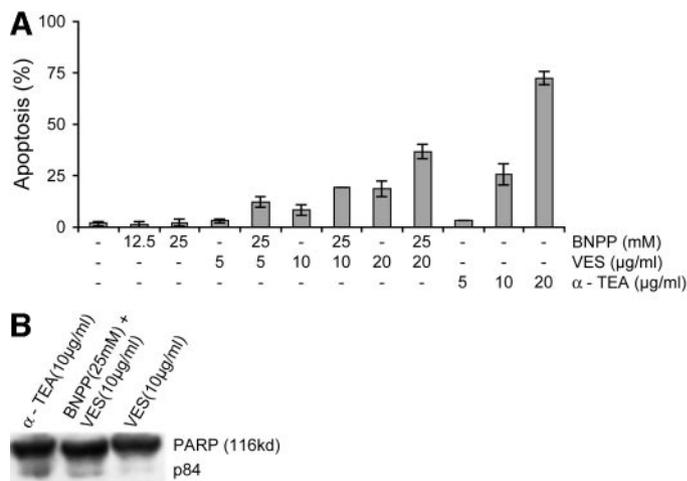


Fig. 6. Cp70 cells at  $1 \times 10^5$  cells/ml in 20-ml flasks were either untreated or pretreated with 12.5 or 25 mM esterase inhibitor bis-(p-nitrophenyl) phosphate (BNPP) for 30 min. The untreated and BNPP-treated cells were then cultured with 5, 10, or 20  $\mu\text{g/ml}$  vitamin E succinate (VES) for 3 days, and cells were 4',6-diamidino-2-phenylindole dihydrochloride stained for analyses of apoptosis as described in "Materials and Methods." Cp70 cells treated with 5, 10, and 20  $\mu\text{g/ml}$  α-TEA served as positive apoptotic control (A). Cell lysates from cp70 cells cultured with 10  $\mu\text{g/ml}$  α-TEA, 10  $\mu\text{g/ml}$  VES, or pretreated with 25 mM for 30 min and then treated with 10  $\mu\text{g/ml}$  VES for 2 days were analyzed by Western immunoblotting for cleavage of caspase 3 substrate poly(ADP-ribose) polymerase (PARP; B). Data depicted in A are mean  $\pm$  SD of three independent experiments. Data in B are representative of multiple experiments.

blotting analyses showing that the caspase-3 substrate PARP is cleaved to the p84 PARP fragment in cell lysates from cp70 cells treated with BNPP +10  $\mu$ g/ml VES to a greater degree than cells treated with 10  $\mu$ g/ml VES alone (Fig. 6B). Cp70 cells treated with 10  $\mu$ g/ml  $\alpha$ -TEA served as a positive control for PARP cleavage (Fig. 6B).

## DISCUSSION

Despite several treatment options, ovarian carcinoma remains the fifth most common cause of cancer-related mortality in women (34). One major problem in the treatment of ovarian cancers has been the development of multidrug resistance. After treatment with various regimens including cisplatin, [*cis*-diamminedichloroplatinum(II)], paclitaxel, and doxorubicin (Adriamycin), many patients experience relapse with drug-resistant tumors and die of chemoresistant disease (35, 36). Thus, there is a need for new anticancer drugs effective against ovarian cancers.

VES is a potent growth inhibitor of a wide variety of human cancer cell lines *in vitro*, as well as an effective tumor growth inhibitor *in vivo* when administered i.p (1, 3–13). Human tumor cells in culture undergo DNA synthesis arrest, differentiation, and apoptosis in response to treatment with VES (reviewed by Refs. 1, 15–21).  $\alpha$ -TEA is a novel vitamin E analogue, which is currently being characterized for its chemotherapeutic and chemopreventive potential.  $\alpha$ -TEA has been shown to decrease tumor burden and reduce lung metastasis in a transplanted syngeneic mouse mammary cancer model (2).

In the studies reported here, we show that  $\alpha$ -TEA is as potent a proapoptotic agent as VES for a number of human breast, prostate, colon, lung, and endometrial cells and markedly better than VES as an inducer of apoptosis in human ovarian and cervical cancer cells. These data suggest that ovarian and cervical cancer cells express sufficient esterase activity to render VES less effective than  $\alpha$ -TEA by hydrolysis of the ester-linked succinate moiety of VES and raise the possibility that these findings may be extended to other gynecological malignancies. Studies comparing the responsiveness of cp70 cells to VES *versus*  $\alpha$ -TEA show that the cisplatin resistant cp70 ovarian tumor cells undergo DNA synthesis arrest but exhibit only low levels of apoptosis when cultured with VES *versus* undergoing both DNA synthesis arrest and apoptosis when cultured with  $\alpha$ -TEA. The potential ramifications of this difference in growth inhibition by VES and  $\alpha$ -TEA were addressed by conducting clonogenic studies, which showed that  $\alpha$ -TEA was superior to VES in preventing cp70 colony formation.

To address the question of why  $\alpha$ -TEA is a more effective proapoptotic agent than VES in ovarian tumor cells, cellular uptake and stability of  $\alpha$ -TEA *versus* VES were examined. Previous studies have suggested that the antitumor activity of VES is associated with the intact molecule. We and others have shown that although VES is capable of inducing DNA synthesis arrest, inducing apoptosis, and preventing colony formation in a variety of cancer cells, neither of the breakdown products, namely RRR- $\alpha$ -tocopherol or succinic acid, exhibit this activity (6, 11, 28, 37–41). Furthermore, Fariss *et al.* (6, 39) have shown that a nonhydrolyzable ether form of VES prevents colony formation of murine leukemia cells and blocks growth of human breast cancer cells, adding additional support that it is the intact molecule that is responsible for the antitumor properties. Thus, the expectation is that cells capable of hydrolyzing VES would not be responsive to VES-mediated antitumor activities. As reported in this article,  $\alpha$ -TEA and VES are taken up by cp70 cells, but most of the intracellular VES is hydrolyzed to RRR- $\alpha$ -tocopherol. In contrast,  $\alpha$ -TEA remains intact in cp70 cells. The HPLC and apoptosis analyses

reported here are the first direct demonstration that when VES is broken down by a cell it loses its capacity to induce apoptosis.

To test the assumption that cellular esterases in the ovarian cp70 cells were responsible for the breakdown of VES, the esterase inhibitor BNPP was used to block esterase activity before VES treatment. Pretreatment of cp70 cells with BNPP resulted in the retention of intact VES and VES proapoptotic effects. Although no references to esterase activity in cp70 cells were not found in the literature, a study by Di Francesco *et al.* (42) showed that the parental A2780 cells were capable of cleaving phenol-ester derivatives, suggesting that these cells contained esterases. Another interesting observation made in the conduct of these studies is that VES retains the ability to induce DNA synthesis arrest in cp70 cells despite the presence of esterases and hydrolysis. One possible explanation is that VES signals DNA synthesis arrest in a rapid, acute manner, thereby initiating a competent signal before hydrolysis, whereas signaling of apoptosis requires a longer, more chronic presence of intact VES.

Mechanism(s) for how VES or  $\alpha$ -TEA induce DNA synthesis arrest in human ovarian cancer cells is not known at present. Studies in human MDA-MB-435 breast cancer cells showed that VES inhibition of DNA synthesis involved a G<sub>0</sub>-G<sub>1</sub> cell cycle block and marked induction of the expression of the cyclin-dependent kinase inhibitor p21 Waf1/Cip1 at the mRNA and protein level (18). Confirmation of the involvement of p21 in this event was demonstrated by the blockage of VES-induced DNA synthesis arrest by antisense oligomers to p21 (18). Furthermore, studies by Turley *et al.* (43) reported that VES inhibited the proliferation of the estrogen receptor-negative human breast cancer cell line BT-20 in the G<sub>1</sub> phase of the cell cycle, at least in part, by inhibition of the transcriptional activity of E2F through negative regulation via cyclin A binding. Additional studies are needed to understand the mechanism(s) of how VES and  $\alpha$ -TEA block DNA synthesis in ovarian cells.

The failure of VES to induce apoptosis in cp70 cells to the same degree as  $\alpha$ -TEA most likely is caused by lower intracellular levels of intact VES because of esterase cleavage of the succinate moiety from VES, resulting in RRR- $\alpha$ -tocopherol that does not exhibit apoptotic inducing properties. Another possibility is that intracellular RRR- $\alpha$ -tocopherol resulting from esterase cleavage of the succinate moiety from VES may block the ability of the remaining intact VES to induce apoptosis by some mechanism that we do not understand at this time. Combination treatments of VES or  $\alpha$ -TEA + RRR- $\alpha$ -tocopherol reduces the level of intracellular VES and  $\alpha$ -TEA and reduces the level of apoptosis induced by these two tocopherol-based compounds. The mechanism(s) whereby RRR- $\alpha$ -tocopherol interferes with intracellular levels of VES and  $\alpha$ -TEA and reduces apoptosis are not understood.

In conclusion, these studies demonstrate that the novel vitamin E analogue,  $\alpha$ -TEA, is a stable compound and a potent inducer of apoptosis in a wide variety of human cancer cells but not normal cells in culture. It is of significance that  $\alpha$ -TEA, a potential chemotherapeutic agent, is structurally stable when administered to human ovarian and cervical cancer cells and acts as a potent proapoptotic agent. Perhaps of more significance, future drug and prodrug design for prevention and treatment of ovarian and cervical cancer need to take into consideration that these cells may express cellular esterases at levels sufficient to cleave critical functional groups.

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## REFERENCES

- Kline K, Yu W, Sanders BG. Vitamin E: mechanisms of action as tumor cell growth inhibitors. *J Nutr* 2001;131:161S–3S.
- Lawson K, Anderson K, Menchaca M, et al. Novel vitamin E analog decreases syngeneic mouse mammary tumor burden and reduces lung metastasis. *Mol Cancer Ther* 2003;2:437–44.
- Prasad KN, Edwards-Prasad J. Effects of tocopherol (vitamin E) acid succinate on morphological alterations and growth inhibition in melanoma cells in culture. *Cancer Res* 1982;42:550–4.
- Prasad KN, Edwards-Prasad J. Vitamin E and cancer prevention: recent advances and future potentials. *J Am Coll Nutr* 1992;11:487–500.
- Schwartz J, Shklar G. The selective cytotoxic effect of carotenoids and alpha-tocopherol on human cancer cell lines *in vitro*. *J Oral Maxillofac Surg* 1992;50:367–73.
- Fariss MW, Fortuna MB, Everett CK, Smith JD, Trent DF, Djuric Z. The selective antiproliferative effects of alpha-tocopherol hemisuccinate and cholesteryl hemisuccinate on murine leukemia cells result from the action of the intact compounds. *Cancer Res* 1994;54:3346–51.
- Kline K, Yu W, Sanders BG. Vitamin E: mechanisms of action as tumor cell growth inhibitors. In: Prasad KN, Cole WC, editors. *Proceeding of the International Conference on Nutrition and Cancer*. Amsterdam: IOS Press; 1998. p. 37–53.
- Neuzil J, Weber T, Gellert N, Weber C. Selective cancer cell killing by alpha-tocopherol succinate. *Br J Cancer* 2000;84:87–9.
- Malafa MP, Neitzel LT. Vitamin E succinate promotes breast cancer tumor dormancy. *J Surg Res* 2000;93:163–70.
- Malafa MP, Fokum FD, Mowlavi A, Abusief M, King M. Vitamin E inhibits melanoma growth in mice. *Surgery (St. Louis)* 2002;131:85–91.
- Neuzil J, Weber T, Schroder A, et al. Induction of cancer cell apoptosis by  $\alpha$ -tocopherol succinate: molecular pathways and structural requirements. *FASEB J* 2001;15:403–15.
- Weber T, Lu M, Andera L, et al. Vitamin E succinate is a potent novel antineoplastic agent with high selectivity and cooperativity with tumor necrosis factor-related apoptosis-inducing ligand (Apo2 ligand) *in vivo*. *Clin Cancer Res* 2002;8:863–9.
- Barnett KT, Fokum FD, Malafa MP. Vitamin E succinate inhibits colon cancer liver metastases. *J Surg Res* 2002;106:292–8.
- Wu K, Shan YJ, Zhao Y, Yu JW, Liu BH. Inhibitory effects of RRR-alpha-tocopherol succinate on benzo(a)pyrene [B(a)P]-induced forestomach carcinogenesis in female mice. *World J Gastroenterol* 2001;7:60–5.
- You H, Yu W, Sanders BG, Kline K. RRR-alpha-tocopherol succinate induces MDA-MB-435 and MCF-7 human breast cancer cells to undergo differentiation. *Cell Growth Differ* 2001;12:471–80.
- You H, Yu W, Munoz-Medellin D, Brown PH, Sanders BG, Kline K. Role of extracellular signal-regulated kinase pathway in RRR-alpha-tocopherol succinate-induced differentiation of human MDA-MB-435 breast cancer cells. *Mol Carcinog* 2002;33:228–36.
- Yu W, Liao QY, Hantash FM, Sanders BG, Kline K. Activation of extracellular signal-regulated kinase and c-Jun-NH2-terminal kinase but not p38 mitogen-activated protein kinases is required for RRR-alpha-tocopherol succinate-induced apoptosis of human breast cancer cells. *Cancer Res* 2001;61:6569–76.
- Yu W, Sanders BG, Kline K. RRR-alpha-tocopherol succinate induction of DNA synthesis arrest of human MDA-MB-435 cells involves TGF-beta independent activation of p21 (Waf1/Cip1). *Nutr Cancer* 2002;43:227–36.
- Yu W, Sanders BG, Kline K. RRR-alpha-tocopherol succinate-induced apoptosis of human breast cancer cells involves Bax translocation to mitochondria. *Cancer Res* 2003;63:2483–91.
- Neuzil J, Weber T, Terman A, Weber C, Brunk UT. Vitamin E analogues as inducers of apoptosis: implications for their potential antineoplastic role. *Redox Report* 2001;6:143–51.
- Neuzil J, Zhao M, Ostermann G, et al. alpha-Tocopherol succinate, an agent with *in vivo* anti-tumour activity, induces apoptosis by causing lysosomal instability. *Biochem J* 2002;362:709–15.
- Yu W, Heim K, Qian M, Simmons-Menchaca M, Sanders BG, Kline K. Evidence for role of transforming growth factor-beta in RRR-alpha-tocopherol succinate-induced apoptosis of human MDA-MB-435 breast cancer cells. *Nutr Cancer* 1997;27:267–78.
- Yu W, Israel K, Liao QY, Aldaz CM, Sanders BG, Kline K. Vitamin E succinate (VES) induces Fas sensitivity in human breast cancer cells: role for Mr 43,000 Fas in VES-triggered apoptosis. *Cancer Res* 1999;59:953–61.
- Shun M-C, Yu W, Gapor A, et al. Pro-apoptotic mechanisms of action of a novel vitamin E analog (alpha-TEA) and a naturally occurring form of vitamin E (delta-tocotrienol) in MDA-MB-435 human breast cancer cells. *Nutr Cancer*. In press 2003.
- Gronlund B, Hogdall C, Hansen H, Engelholm SA. Results of reinduction therapy with paclitaxel and carboplatin in recurrent epithelial ovarian cancer. *Gyn Oncol* 2001;83:128–34.
- Israel K, Yu W, Sanders BG, Kline K. Vitamin E succinate induces apoptosis in human prostate cancer cells: role for Fas in vitamin E succinate-triggered apoptosis. *Nutr Cancer* 2000;36:90–100.
- Behrens B, Hamilton T, Masuda H, et al. Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987;47:414–8.
- Carini R, Poli G, Dianzain MU, Maddix SP, Slater TF, Cheesman KH. Comparative evaluation of the antioxidant activity of alpha-tocopherol, alpha-tocopherol polyethylene glycol 1000 succinate and alpha-tocopherol succinate in isolated hepatocytes and liver microsomal suspensions. *Biochem Pharmacol* 1990;39:1597–601.
- Charpentier A, Groves S, Simmons-Menchaca M, et al. RRR-alpha-tocopherol succinate inhibits proliferation and enhances secretion of transforming growth factor-beta (TGF-beta) by human breast cancer cells. *Nutr Cancer* 1993;19:225–39.
- Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer Res* 1993;53:3976–85.
- Hall EJ. Cell survival curves. In: Hall EJ, editor. *Radiobiology for the radiologist*, 5th ed. Philadelphia: Lippincott, Williams, and Wilkins; 2000. p. 32–50.
- Tirmenstein MA, Watson BW, Haar NC, Fariss MW. Sensitive method for measuring tissue alpha-tocopherol and alpha-tocopheryloxybutyric acid by high-performance liquid chromatography with fluorometric detection. *J Chromatogr B Biomed Sci Appl* 1998;707:308–11.
- Koshkina NV, Glibert BE, Waldrep JC, Seryshev A, Knight V. Distribution of camptothecin after delivery as a liposome aerosol or following intermuscular injection in mice. *Cancer Chemother Pharmacol* 1999;44:177–86.
- Jemal A, Tiwari RC, Murray T, et al, editors. *Cancer statistics, 2004*. CA - Cancer J Clin 2004;54:8–29.
- McGuire WP, Ozols RF. Chemotherapy of advanced ovarian cancer. *Semin Oncol* 1998;25:340–8.
- Albert DS. Treatment of refractory and recurrent ovarian cancer. *Semin Oncol* 1999;26(Suppl):8–14.
- Qian M, Kralova J, Yu W, et al. c-Jun involvement in vitamin E succinate induced apoptosis of reticuloendotheliosis virus transformed avian lymphoid cells. *Oncogene* 1997;15:223–30.
- Turley JM, Sanders BG, Kline K. RRR-alpha-tocopherol succinate modulation of human promyelocytic leukemia (HL-60) cell proliferation and differentiation. *Nutr Cancer* 1992;18:201–13.
- Djuric Z, Heilbrun LK, Lababidi S, Everett-Bauer CK, Fariss MW. Growth inhibition of MCF-7 and MCF-10A human breast cells by alpha-tocopherol hemisuccinate, cholesteryl hemisuccinate and their ether analogs. *Cancer Lett* 1997;111:133–9.
- Yu W, Sanders BG, Kline K. RRR-alpha-tocopherol succinate inhibits EL4 thymic lymphoma cell growth by inducing apoptosis and DNA synthesis arrest. *Nutr Cancer* 1997;27:92–101.
- Yu W, Simmons-Menchaca M, Gapor A, Sanders BG, Kline K. Induction of apoptosis in human breast cancer cells by tocopherols and tocotrienols. *Nutr Cancer* 1999;33:26–32.
- Di Francesco AM, Hargreaves RH, Wallace TW, et al. The abnormal cytotoxicities of 2,5-diaziridinyl-1,4-benzoquinone-3-phenyl esters. *Anticancer Drug Des* 2000;15:347–59.
- Turley JM, Ruscetti FW, Kim S-J, Fu T, Gou FV, Birchenall-Roberts MC. Vitamin E succinate inhibits proliferation of BT-20 human breast cancer cells: Increased binding of cyclin A negatively regulates E2F transactivation activity. *Cancer Res* 1977;37:2668–75.

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## Differential Response of Human Ovarian Cancer Cells to Induction of Apoptosis by Vitamin E Succinate and Vitamin E Analogue, $\alpha$ -TEA

Kristen Anderson, Marla Simmons-Menchaca, Karla A. Lawson, et al.

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