ABSTRACT Vitamin E is a term that describes a group of compounds with similar yet unique chemical structures and biological activities. One interesting property possessed by certain vitamin E compounds—namely, δ-tocotrienol, RRR-α-tocopheryl succinate [vitamin E succinate (VES), a hydrolyzable ester-linked succinic acid analogue of RRR-α-tocopherol], and a novel vitamin E analogue referred to as α-TEA (α-tocopherol ether linked acetic acid analogue, which is a stable nonhydrolyzable analogue of RRR-α-tocopherol)—is their ability to induce cancer cells but not normal cells to undergo a form of cell death called apoptosis. In contrast, the parent compound, RRR-α-tocopherol, also referred to as natural or authentic vitamin E and known for its antioxidant properties, does not induce cancer-cell apoptosis. Efforts to understand how select vitamin E forms can induce cancer cells to undergo apoptosis have identified several nonantioxidant biological functions, including restoration of pro-death transforming growth factor-β and Fas signaling pathways. Recent studies with α-TEA show it to be a potent inducer of apoptosis in a wide variety of epithelial cancer cell types, including breast, prostate, lung, colon, ovarian, cervical, and endometrial in cell culture, and to be effective in significantly reducing tumor burden and metastasis in a syngeneic mouse mammary tumor model, as well as xenografts of human breast cancer cells. Studies also show that α-TEA, in combination with the cyclooxygenase-2 inhibitor celecoxib and the chemotherapeutic drug 9-nitro-camptothecin decreases breast cancer animal model tumor burden and inhibits metastasis significantly better than do single-agent treatments. J. Nutr. 134: 3458S–3462S, 2004.

KEY WORDS: vitamin E • cancer • chemotherapy • tumor burden • metastasis

Vitamin E is a general term used indiscriminately to refer to a group of 8 different naturally occurring compounds known as tocopherols and tocotrienols, as well as synthetic vitamin E (a chemical mixture composed of 12.5% authentic RRR-α-tocopherol and 87.5% stereoisomers; namely, 7 molecules produced during the manufacturing process that have the same number and types of atoms found in RRR-α-tocopherol linked in the same order but differing in their spatial arrangement) (1,2). Thus, natural authentic vitamin E (referred to as RRR-α-tocopherol or δ-α-tocopherol) and synthetic vitamin E (referred to as all-α-ethyl-α-tocopherol or dl-α-tocopherol) are not chemically equivalent (Fig. 1). RRR-α-tocopherol, the most prevalent form in the human body, has traditionally been recognized as a free radical-scavenging antioxidant important for protecting polyunsaturated fats from peroxidation, whose deficiency impairs mammalian fertility (1). Research suggesting that certain forms of vitamin E exhibit key nonantioxidant functions and data showing RRR-α-tocopherol to be selectively incorporated into the body over other biologically active vitamin E forms requires reevaluation of the role of vitamin E in health and disease processes (3–8).

Both authentic RRR-α-tocopherol and synthetic vitamin E can be purchased as acetate or succinate derivatives. These modifications to the chroman head of RRR-α-tocopherol are performed to protect the hydroxyl moiety at the C-6 position from oxidation when exposed to air and must be removed to restore antioxidant potential (2).

Recently, our laboratory developed a nonhydrolyzable ether analogue of RRR-α-tocopherol, referred to as α-TEA (α-tocopherol ether linked acetic acid analogue of RRR-α-tocopherol) (Fig. 1). α-TEA is made from RRR-α-tocopherol by attaching an acetic acid moiety to the phenolic oxygen at carbon 6 of the chroman head by a nonhydrolyzable ether linkage. α-TEA differs from the succinate and acetate derivatives not only in the attached moiety but also in the linkage; namely, a nonhydrolyzable ether linkage vs. a hydrolyzable ester linkage for the succinic acid and acetate forms (9). Thus, no antioxidant properties are expected for the intact α-TEA analogue. Both vitamin E succinate (VES) and α-TEA have been demonstrated to exhibit anticancer properties (2,9–15).

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3 Abbreviations used: α-TEA, α-tocopherol ether linked acetic acid analog; 9-NC, 9-nitro-camptothecin; TGF-β, transforming growth factor-β; VES, vitamin E succinate.
Despite intense study and great advances, causes, effective treatments, and prevention of cancers remain elusive. Deaths from cancer in both males and females is second only to deaths from heart disease (16). Breast and prostate cancers are the cancers with highest incidence in females and males, and these cancers are second only to lung cancer in causing death (16). Resistance to therapy is a hallmark of advanced cancers.

Cancers are clonal in origin, originating from normal cells that undergo multiple genetic changes, rendering survival advantage. Despite the enormous complexities involved in the
conversion of normal cells to cancer cells, selection for survival appears to be a common event among cancers. Evolving cancer cells do not seem to follow one set pattern; rather, cancer development is a multistep process favoring genetic modifications that confer cell survival. Advanced cancers that acquire growth factor independence and resistance to apoptosis exhibit further survival advantage (17). Studies in our laboratory show that the acquisition of resistance to apoptosis by breast, ovarian, and prostate cancer cells are reversible events, with vitamin E compounds α-TEA and VES inducing the cancer cells to undergo cell death by restoration of pro-death signaling pathways (10,15,18–22).

The role of diet and nutrition in cancer prevention and treatment was extensively covered at the American Institute for Cancer Research Conference on Food, Nutrition, and Cancer in 2003 (23,24). Specific nutrients and dietary constituents are known to be important players in cancer prevention and treatment (25,26). It is becoming increasingly clear that treatment of aggressive cancers that have metastasized to distant secondary sites is a daunting task, and expectations that a single agent will eliminate such cancers are not realistic. The focus of our studies is on vitamin E induced cell death by apoptosis of human breast cancer cells via restoration of pro-death signaling pathways.

Objectives of this review are to summarize what is currently known about 1) the potential of vitamin E as a chemopreventive agent; 2) the efficacy of the vitamin E derivative α-TEA, alone and in combination with established therapeutic drugs, as an anticancer agent for reduction of tumor burden and metastasis, using a syngeneic murine mammary cancer model as well as a human xenograft model involving human breast cancer cells; and 3) mechanisms of action of vitamin E compounds in tumor burden reduction and inhibition of metastasis.

Vitamin E chemopreventive potential

Although vitamin E is a popular supplement marketed for its potential beneficial antioxidant effects for a number of chronic diseases, including various forms of cancer, a Food and Nutrition Board panel on Dietary Reference Intakes for vitamin C, vitamin E, selenium, and carotenoids concluded that observational epidemiological studies provide only limited evidence for a protective association of vitamin E with lung cancer (1). Furthermore, the Food and Nutrition Board concluded that data from randomized clinical trials, which are considered the gold standard for health research, are suggestive for the ability of vitamin E to prevent prostate cancer but caution that this information is from a single trial that needs to be confirmed and that this study was not designed to examine the correlation between vitamin E and prostate cancer (1).

Two extensive reviews of epidemiologic studies (observational studies and intervention trials) investigating various relationships between vitamin E and breast cancer, including vitamin E dietary intake, vitamin E serum levels, or α-tocopherol levels in subcutaneous adipose tissue, were published recently (27,28). Schwenke (28) concluded that observational studies that analyzed dietary exposure to RRR-α-tocopherol with or without the presence of other tocopherols or tocotrienols suggests that vitamin E from dietary sources may modestly protect women from breast cancer; however, there was no evidence that vitamin E supplements conferred any protection against breast cancer. The popular antioxidant hypothesis proposes that antioxidant nutrients protect against chronic diseases by decreasing oxidative damage. However, this hypothesis has neither been proven nor consistently supported by the findings of published intervention trials (29).

Preventive studies using chemically induced mammary cancer in Sprague Dawley rats and dietary supplementation of vitamin E—adequate diets with various forms of vitamin E concluded that there was only limited or no evidence for a protective effect (2,27,28,30).

Compelling data to support natural vitamin E (RRR-α-tocopherol) as a chemopreventive agent are lacking. Preclinical data in the literature are inconclusive, and RRR-α-tocopherol failed to reduce the risk of cancer in large-scale clinical trials (1). Because RRR-α-tocopherol is preferentially taken up in the liver by binding to the α-tocopherol transfer protein, this raises the question as to whether RRR-α-tocopherol may actually prevent the anticancer properties of other forms of vitamin E, perhaps by limiting their bioavailability. Studies by Regina Brigelius-Flohe and co-workers (University of Potsdam, Germany, unpublished results, 2004) showed that uptake of RRR-α-tocopherol and RRR-γ-tocopherol are at the same rate for the first 6 h after supplementation in humans but that γ-tocopherol is then rapidly metabolized. Studies in human ovarian A2780/cp70 cancer cells showed that both VES or α-TEA are taken up by the tumor cells when administered in combination with RRR-α-tocopherol, but VES and α-TEA levels in the cotreated cells are lower than in singly treated cells (12). These studies further 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 numbers of apoptotic cells in comparison to VES and α-TEA treatments alone (12).

In another study, compared with placebo control, supplementation of humans with 400 IU/d of RRR-α-tocopheryl acetate for 2 mo enhanced RRR-α-tocopherol serum levels by 32%, reduced serum RRR-γ-tocopherol concentrations by 54%, and reduced detectable levels of RRR-δ-tocopherol in individuals by 72% (31). This information, taken together with data that suggest that γ-tocopherol may be a more potent cancer chemopreventive agent than α-tocopherol (32,33), along with the fact that vitamin E supplementation is common practice suggests that there is a potential for RRR-α-tocopherol interference with anticancer effectiveness of other vitamin E compounds, perhaps by limiting their bioavailability. This possibility deserves further investigation.

Development and evaluation of α-TEA in rodent models as an anticancer agent

Approximately 50 vitamin E analogs were synthesized and screened for their ability to induce human tumor cells in culture to undergo apoptosis. Eleven vitamin E analogs exhibited potent anticancer properties, and α-TEA was selected as the lead vitamin E compound for further development (Fig. 1). Because α-TEA is a very hydrophobic compound, it was incorporated into liposomes to make it more water soluble. In addition to oral delivery, aerosol delivery was investigated, because this route has proved very effective for liposome-formulated compounds (9).

Liposome-formulated α-TEA administered to BALB/c mice by aerosol (36 μg deposited into respiratory tract daily) for 17 d significantly reduced subcutaneously injected 66 cl-4-GFP mouse mammary tumor cell growth and lung metastasis (9). Tumor volume was reduced by 65% in comparison with the aerosol control (P < 0.001). Aerosol and untreated controls exhibited 40% visible lung metastases; whereas mice treated with α-TEA had no visible lung metastases (9). Lungs from aerosol control mice exhibited an average of 60 microscopic
fluorescent green lung lesions, whereas mice treated with α-TEA exhibited an average of 11 fluorescent microscopic lesions (9). In summary, these studies show α-TEA to be an effective anticancer agent when used singly.

Liposome-formulated α-TEA (36 µg deposited into respiratory track daily) or 9-nitro-camptothecin (9-NC; 0.4 µg deposited into respiratory track daily) administered to BALB/c mice by aerosol separately or in combination for 21 d reduced subcutaneously injected 66 cl-4-GFP mouse mammary tumor growth and lung metastasis (13). The combination of α-TEA and 9-NC significantly reduced (P < 0.001) growth of tumors. The incidence of visible lung metastasis was 83% in control vs. 8% in the α-TEA, 9-NC, and combination treated groups. Likewise, lungs and lymph nodes showed a statistically significant decrease in fluorescent microscopic lesions for the separate treatments, as well as the combination treatment. Analyses of primary tumor size for proliferation and apoptosis showed the separate and combination treatment groups to have lower numbers of proliferating cells and elevated numbers of apoptotic cells (13).

Liposome-formulated α-TEA administered daily by aerosol (36 µg deposited into respiratory tract daily) and celecoxib administered daily by diet (500 or 1250 mg/kg), separately or in combination for 31 d reduced tumor growth of human MDA-MB-435 breast cancer cells transplanted into immune compromised NU/NU mice in comparison with control (P < 0.001 for all treatment groups) (14). The combination treatment of α-TEA plus celecoxib (1250) reduced tumor volume significantly better than either single treatment (P < 0.001 and P < 0.001, respectively) (14). The mean number of visible lung metastases was significantly reduced in all treatment groups (except for the celecoxib 500 treatment group) in comparison with control (14). The mean number of fluorescent lung and lymph-node metastases in all treatment groups was significantly lower than in the control group. Furthermore, the mean number of microscopic lung metastases in the α-TEA plus celecoxib (1250) group was significantly lower than for either separate treatment (14).

Taken together, these rodent model studies suggest that α-TEA is an effective anticancer agent both alone and in combination with other established anticancer agents.

**Mechanisms whereby α-TEA and VES inhibit cancer**

Our mechanistic studies have focused primarily on the mechanisms whereby VES inhibits the growth of human breast cancers in culture by induction of DNA synthesis arrest, cellular differentiation, and apoptosis (7,18,21,34,35). Inhibition of cell proliferation involves a G0/G1 cell-cycle block, mediated in part by mitogen-activated protein kinases MEK1 and ERK1 and upregulation of the key cell-cycle regulatory protein p21(waf1/cip1)) (21). Induction of differentiation is characterized by morphological changes, elevated beta casein mRNA expression, expression of milk lipids, elevated cytokeratin 18 protein, and downregulation of Her2/neu protein expression (34). Differentiation is mediated in part by activation of MEK1, ERK1/2, and phosphorylation of the transcription factor c-Jun (35).

Of the multiple apoptotic signaling events modulated by VES, especially noteworthy are its ability to convert Fas/Fas ligand nonresponsive human breast cancer cells to Fas/Fas ligand responsiveness and to convert transforming growth factor-β (TGF-β) nonresponsive breast cancer cells to TGF-β responsiveness, with both restored signaling pathways converging on prolonged activation of JNK/c-Jun, followed by translocation of Bax protein to the mitochondria, induction of mitochondria permeability transition, followed by cytochrome c release into the cytoplasm, activation of caspases 9 and 3, cleavage of poly (ADP-ribose) polymerase, and apoptosis (36). Although the mechanisms whereby α-TEA induces human MDA-MB-435 breast cancer cells to undergo apoptosis have not been studied as extensively, studies thus far show α-TEA to mimic VES. Treatment of MDA-MB-435 breast cancer cells with α-TEA restores both Fas/Fas ligand and TGF-β signaling pathways, which converge on JNK, followed by induction of apoptosis (15).

**Summary**

Vitamin E describes a number of compounds that differ in chemical structure and biological activity. Of the vitamin E forms, δ-tocopherol; α-, γ-, and δ-tocotrienol; and derivatives VES and α-TEA selectively induce cancer cells to undergo apoptosis. Although progress in characterizing the antitumor functions of vitamin E forms has been made, much remains to be done. Comparative analyses of individual vitamin E compounds, separately and in combination, in preclinical animal models deserve further investigation.

**LITERATURE CITED**


