Vitamin E: Mechanisms of Action as Tumor Cell Growth Inhibitors$^{1,2}$

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Vitamin E is an essential fat-soluble vitamin that functions, at least in part, as a lipid-soluble antioxidant. 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 epidemiologic studies provide only limited evidence for a protective association of vitamin E with lung cancer and that data from intervention trials are most suggestive for the ability of vitamin E to prevent prostate cancer; however, the panel cautioned that this information is from a single trial that must be confirmed and that the study was not designed to examine the correlation between vitamin E and prostate cancer (Institute of Medicine 2000). Results from preclinical studies using various experimental animals models have been inconsistent in detecting anticancer effects of vitamin E (Kimmick et al. 1997, Prasad and Edwards-Prasad 1992).

Structure and nomenclature of vitamin E compounds

Vitamin E is a general term used indiscriminately to refer to a group of naturally occurring compounds called tocopherols and tocotrienols as well as synthetic vitamin E, and acetate and succinate derivatives of both natural and synthetic α-tocopherol (Fig. 1) (Institute of Medicine 2000, Kamal-Eldin and Appelqvist 1996). Vitamin E acetate and succinate derivatives are often used in vitamin E supplements because of their increased stability in air, but these compounds are not active antioxidants unless the esterification at the C-6 position is reversed and the free phenol (—OH) is regenerated. Further investigations have demonstrated that vitamin E activity in animal reproduction tests (rat resorption-gestation tests). Such measurements are not accurate measures of other recognized or postulated vitamin E biological activities such as various antioxidant functions (inhibition of lipid peroxidation or inhibition of nitric acid species formation), cholesterol and thromboxane suppressive actions and proapoptotic activity. Bioavailability is also an important issue (Traber 2000). Although it is clear that RRR-α-tocopherol is transported preferentially via endogenous fat transport by an α-tocopherol transfer protein in the liver, the status of absorption of other vitamin E compounds and bioavailability to tissues during exogenous fat transport remain to be examined fully.

In summary, to help clarify the role of vitamin E in cancer prevention or therapy, future studies must use appropriate chemical forms of vitamin E that exhibit anticancer biological activities and are formulated and administered to achieve necessary intracellular levels. To date, only the tocotrienols and the succinced form of RRR-α-tocopherol [RRR-α-tocopheryl succinate or vitamin E succinate (VES)] have been reported to exhibit potent antiproliferative properties in studies of human tumor cells in culture (Djuric et al.1997, Guthrie et al. 1991, Israel et al. 2000; Kline et al. 1998, Nesaretnam et al. 1998, Turley et al.1997, Yu et al. 1999a and 1999b).

Apoptotic activity of vitamin E compounds

Several mechanisms that explain how vitamin E compounds might produce beneficial protective effects in cancer have been postulated, including the following: inhibition of cancer formation by the quenching of free radicals; direct effects on tumor cells such as control of tumor growth through induction of differentiation; cell cycle inhibition or induction of apoptosis; and elimination of tumor cells by increased efficacy of antitumor actions by the immune system (Kelloff et al. 1994, Prasad and Edwards-Prasad 1992, Theriault et al. 1999). A comparison of the apoptosis-inducing properties of vitamin E compounds revealed that α-, δ- and γ-tocotrienols (β-tocotrienol was not available for study) and VES are the most potent inducers of apoptosis in human breast cancer cells in culture (Yu et al. 1999b).

Vitamin E succinate induction of apoptosis of human cancer cells

Turley and co-workers (1995) were the first to report that VES induced apoptosis in human tumor cells (B lymphoma) in culture. Further investigations have demonstrated that VES is a potent inducer of apoptosis for a wide range of human cancer cells of both epithelial and lymphoid origin, tumor cell types

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* Abbreviations used: ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; Smads, mediators of TGF-β signaling; TGF-β, transforming growth factor-β; VES, vitamin E succinate.
that account for >90% of all human malignancies. VES at a 50% effective concentration range of 5–10 mg/L is capable of inducing human breast (MCF-7, MDA-MB-231, MDA-MB-435, SKBR-3), cervical (ME-180), endometrial (RL-95–2), lung (A549) and lymphoid (Raji, Ramos, Jurkat, HL-60, RLB-lymphoma) cells to undergo apoptosis (Israel et al. 2000, Kline et al. 1998, unpublished data). In contrast, VES does not induce normal human mammary epithelial cells or normal human prostatic epithelial cells to undergo apoptosis (Israel et al. 2000, Yu et al. 1999a). VES induces apoptosis in a concentration- and time-dependent manner. For example, VES at 5, 10 and 20 mg/L for 3 d induces ~10, 48 and 70% of human breast cancer cells (MDA-MB-435) to undergo apoptosis, respectively; VES at 10 mg/L induces ~8, 19, 50 and 75% of MDA-MB-435 cells to exhibit terminal-stage apoptotic characteristics in 1, 2, 3 and 4 d, respectively (Kline et al. 1998).

**Signal transduction events in VES-induced tumor cell apoptosis**

Studies show that VES is inducing human MDA-MB-435 breast cancer cells to undergo apoptosis via involvement of at least three signaling pathways, i.e., transforming growth factor-β (TGF-β), Fas (CD95/APO-1), and the c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) signaling pathways (Yu et al. 1997, 1998 and 1999a). Breast cancer cells exhibit alternations in both TGF-β and Fas signaling, with VES having the unique ability to restore tumor cell responsiveness to both exogenous TGF-β ligand-activated and agonistic anti-Fas antibody-triggered apoptosis. The critical roles of these signaling pathways for VES-induced apoptosis have been demonstrated by various functional knockout approaches including the following: 1) blocking antibodies to TGF-β ligands and Fas receptor; 2) chemical inhibition of TGF-β ligand activation and caspase activity; 3) antisense blockage of TGF-β receptor II, TGF-β1 ligand and c-Jun; and 4) dominant negative blockage of c-Jun (Israel et al. 2000, Yu et al. 1997, 1998 and 1999a).

**Transforming growth factor-β signaling pathway.** TGF-βs are the prototype of a large family of structurally related factors that mediate a wide spectrum of biological responses, including growth inhibition and apoptosis of epithelial cells (Massague 1998). The TGF-β signaling pathway is dysfunctional in MDA-MB-435 cells but is restored by treatment with VES via conversion of latent, biologically inactive TGF-β to the active ligand and up-regulation of TGF-β receptor II expression on the cell surface membrane. To appreciate the importance of the fact that VES can restore TGF-β signaling in TGF-β nonresponsive cancer cells, it is important to understand the role TGF-β signaling plays in tumorigenesis (Brattain et al. 1996). During malignant progression, tumor cells may become insensitive to the growth-inhibitory effects of TGF-β and exhibit uncontrolled proliferation. The mechanisms of resistance to TGF-β antiproliferative effects are not fully understood but downstream-regulation of both ligand and receptor is frequently observed. Treatment of MDA-MB-435 cells activates the ligand and up-regulates membrane TGF-β receptor II levels. However, instead of inducing growth inhibition via the Smad signaling pathway, VES restoration of TGF-β signaling results in apoptosis via activation of the JNK signaling pathway.

**Fas/APO-1/CD95 signaling pathway.** Fas (CD95/APO-1) is a member of the death receptor subfamily of the tumor necrosis factor receptor–nerve growth factor receptor superfamily. CD95 mediates apoptosis when triggered by agonistic antibodies or its oligomerizing ligand (Fas ligand), expressed on cell surface membranes or in a soluble form (Peter and Krammer 1998). VES restores Fas signaling to Fas-insensitive human breast cancer cells by restoring Fas to the cell surface membrane and up-regulating the expression of Fas ligand (Israel et al. 2000, Turley et al. 1997, Yu et al. 1999a). To appreciate the importance of the fact that VES can restore Fas signaling in Fas nonresponsive cancer cells, it is important to understand the role Fas signaling plays in tumorigenesis (Hug 1997). Tumor cell resistance to apoptosis is common and may be fundamentally important to tumor progression. Intracellular sequestration of Fas death receptors causes cancer cells to exhibit resistance to a variety of apoptotic inducers, including chemotherapeutic drugs.

**Mitogen-activated protein kinase signaling pathway.** MAPKs are components of pathways that relay signals to the cell nucleus in response to a diverse array of extracellular stimuli (Ip and Davis 1998). Typical MAPK cascades (stimuli-GTPase-MAPK4-MAPK3-MAPK2-MAPK) are involved in the control of a wide spectrum of cellular processes including growth, differentiation, survival and death. Activated MAPKs [ERK, JNK, p38] translocate to the nucleus, phosphorylate substrates including transcription factors and thereby control cell fates such as proliferation, cell cycle arrest, differentiation or death. VES induced TGF-β and Fas signal apoptosis via JNK in human breast cancer cells. **Ongoing studies**. Ongoing studies are focused on further investigations of VES-induced apoptosis, including investigations of vitamin E binding proteins and signaling events upstream of JNK and ERK. Our current hypothesis concerning how VES induces apoptosis is depicted in Figure 2. VES triggers the TGF-β signaling pathway via activation of latent TGF-β and up-regula-

![Figure 1](https://academic.oup.com/jn/article-abstract/131/1/161S/4686494)

**Figure 1** Structures of tocopherols, tocotrienols and RRR-α-tocopheryl succinate (vitamin E succinate). For both tocopherols and tocotrienols: α: R1 = CH3, R2 = CH3; β: R1 = CH3, R2 = H; γ: R1 = H, R2 = CH3; δ: R1 = H, R2 = H. The tocopherols have a saturated phytyl side chain. The tocotrienols have an unsaturated isoprenoid side chain. α-Tocopherol has a 2R, 4R, 8R configuration, abbreviated RRR, due to the chiral centers at C-2, C-4 and C-8. Synthetic vitamin E (not pictured) consists of equal amounts of eight stereoisomers with the configurations RRR, RRS, RSR, SRR, SSR, SRS and SSS. RRR-α-tocopheryl succinate, a derivative of RRR-α-tocopherol, has an ester-linked succinic acid moiety attached at the C-6 position.
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FIGURE 2 Model of signaling events in vitamin E succinate (VES)-induced apoptosis in human breast cancer cells. Known signaling events are boxed in gray. Abbreviations: TGF-β, transforming growth factor-β; FADD, Fas-associated death domain-containing molecule; Ras, Rac, Cdc42, small guanosine triphosphate binding proteins; Smads, mediators of TGF-β signaling; Smad, Sma- and Mad-related proteins; TAK, TGF-β receptor II; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MPT, mitochondrial permeability transition; AIF, apoptosis inducing factor.

LITERATURE CITED


SUMMARY

Vitamin E is a generic term used to describe a number of compounds that differ in chemical structure and biological activity. One interesting property that certain vitamin E compounds, namely, the tocotrienols and the vitamin E derivative, VES, possess is the ability to induce cancer cells but not normal cells to undergo apoptosis. Investigations of mechanisms of action of these compounds, to date primarily VES, are helping to increase basic knowledge about apoptotic signal transduction in cancer cells in general. It is hoped that understanding basic structure-function relationships will lead to the design of new synthetic agents with improved ability to kill cancer cells.