Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs

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Current observations in the literature suggest that vitamin E may be a suitable candidate for the adjuvant treatment of cancer. Even though historically most research focused on α-tocopherol, more recent evidence suggests that the other isomers of vitamin E (β-, γ- and δ-tocopherols and α-, β-, γ- and δ-tocotrienols) differ in their proapoptotic potencies. The main focus of this communication is the current understanding of the molecular mechanisms regulated by vitamin E isomers and their analogs during the induction of apoptosis. This review highlights that the mitochondria are the major target for the induction of apoptosis by vitamin E isomers and analogs and that the various signaling pathways regulated by these agents are likely to contribute towards maximizing the intrinsic pathway of apoptosis triggered initially by the mitochondria. Overall, the presentation of recent studies from the literature in this communication allows the drawing of the following important conclusions: (i) a direct link exists between the antioxidant activity of each isomer/derivative and proapoptotic potency, (ii) tocotrienols are more effective proapoptotic agents than tocopherols, (iii) synthetic modifications of the naturally occurring compounds improve their apoptotic potency and (iv) vitamin E isomers and derivatives regulate caspase-independent pathways of apoptosis. The latter combined with the evidence presented in this review regarding the additive or synergistic anticarcinogenic effects obtained when vitamin E analogs are used in combination with other cancer chemotherapeutic agents, supports further research to design the most promising vitamin E derivatives and clinically test them in adjuvant chemotherapeutic treatments.

Key words: vitamin E; tocopherol; tocotrienol; apoptosis; α-tocopherol succinate; cancer chemoprevention; chemotherapy

Vitamin E is an important micronutrient essential for preserving the balance between antioxidant and prooxidant reactions in tissues. Vitamin E is relatively nontoxic and well tolerated by humans. Some reported side effects are minor in nature and include gastrointestinal symptoms, dermatitis and fatigue. Vitamin E exists in nature as 8 isomers, i.e., 4 tocopherols (RRR-α, RRR-β, RRR-γ and RRR-δ tocopherols, abbreviated as α-TOC, β-TOC, γ-TOC and δ-TOC respectively) and 4 tocotrienols (RRR-α, RRR-β, RRR-γ and RRR-δ tocotrienols, abbreviated as α-TT, β-TT, γ-TT and δ-TT, respectively), and all isomers have strong antioxidant activities. Tocopherols are most commonly found in nuts and vegetable oils, whereas tocotrienols are primarily derived from palm oil, oat, rye, wheat germ, barley and rice bran. Structurally, vitamin E consists of a chroman head with 2 rings (1 phenolic acid and 1 heterocyclic) and either a saturated (tocopherols) (Fig. 1) or an unsaturated (tocotrienols) (Fig. 2) phytyl tail. Tocopherols and tocotrienols differ in the number of methyl groups present on the chroman head (Figs. 1 and 2). Vitamin E isomers consist of 3 moieties. The Functional Moiety (I) comprises the redox-active hydroxyl group in all tocopherols and tocotrienols, and this group can be modified to produce tocopheryl or tocotrienyl derivates, e.g., α-tocopherol is esterified with a sucfinoyl moiety to produce α-tocopheryl succinate (α-TOS) (Fig. 3). As it will be discussed in the following sections, it is currently believed that Moiety (I) is responsible for the apoptotic properties of vitamin E derivatives. The Stabilizing Moiety (II) modulates certain signaling pathways, such as the protein phosphate 2/protein kinase C pathway, whereas the Hydrophobic Moiety (III) is responsible for docking the compounds in circulating lipoproteins and in biological membranes.

Emerging evidence in the literature suggests that vitamin E has anticarcinogenic activities and may be a suitable candidate for the adjuvant treatment of cancer. The chemopreventive properties of vitamin E were first suspected when studies showed that people in the Mediterranean area, who consume diets rich in vitamin E isoforms, have a lower risk of colon cancer than people in Northern Europe and USA. Further support for the possible use of vitamin E in cancer chemoprevention came from another study, which showed that low nutritional intake of vitamin E increases prostate cancer risk supporting the possible use of this nutrient in the chemoprevention of prostate cancer.

The α-tocopherol, β-carotene Cancer Prevention (ATBC) Study was the first large scale epidemiological study showing that vitamin E may play an important role in the prevention of prostate cancer.
In this study, the investigators examined whether daily supplementation with 50 mg \( \alpha \)-tocopherol acetate and/or 20 mg \( \beta \)-carotene reduced the incidence of or mortality from oral/pharyngeal, esophageal and laryngeal cancers. The results of this study did not provide support for a protective effect of vitamin E supplementation on upper aerodigestive tract cancers. However, dietary treatment with vitamin E demonstrated a 32% reduction in the incidence of prostate cancer when compared to men who did not receive vitamin E.\(^{12,13}\) In addition, further studies revealed that vitamin E may delay cancer progression in patients with prostate cancer,\(^{14,15}\) whereas another study showed that higher circulating concentrations of \( \alpha \)-TOC within the normal range was associated with significantly lower total and cause specific mortality in order male smokers.\(^{16}\) The chemopreventive properties of vitamin E were also revealed by the Melbourne Colorectal Cancer Study, where it was shown that dietary vitamins E and C were statistically significantly protective for both colon and rectal cancer, and that for both vitamins there was a dose-response effect of increasing protection particularly so for colon cancer.\(^{17}\)

The ongoing NIH-sponsored Selenium and Vitamin E Cancer Prevention Trial (SELECT) is investigating selenium and vitamin E for prostate cancer prevention based on encouraging results from earlier studies. SELECT, sponsored by the National Cancer Institute, is an intergroup Phase III, randomized, double-blind, placebo-controlled, population-based clinical trial designed to test the efficacy of selenium and vitamin E alone and in combination in the prevention of prostate cancer. Enrollment for SELECT began in 2001 and the final results are anticipated in 2013.\(^{18}\)

The promising results in the literature regarding the chemopreventive properties of vitamin E led to the further investigation of the anticancer properties of not only \( \alpha \)-TOC but also of the other isomers of vitamin E. Studies using cancer cell lines, animal models and clinical trials have shown that vitamin E isomers have cytotoxic and anticancer activities.\(^{19-25}\) In addition, in the past few years, most research has focused towards structural variations mainly within the functional moiety of vitamin E with the aim to enhance the proapoptotic potency of these agents.

In this communication, we examined the data available in the literature relating to the specific anticarcinogenic properties of each of the different naturally occurring vitamin E isomers and their newly synthesized derivatives. Furthermore, we analyzed the role of mitochondria and investigated the main signaling mechanisms involved in the induction of apoptosis by vitamin E. Lastly, we examined the role of cellular microenvironment and explored the data existing in the literature supporting the induction of caspase-independent apoptosis by vitamin E and its analogs.

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**Figure 1** – The structures of tocopherols. The molecules comprise 3 major moieties, the Functional Moiety (I), the Signaling Moiety (II) and Hydrophobic Moiety (III), and they differ on the number of methyl groups present on the chroman head.

**Figure 2** – The structures of tocotrienols. The molecules comprise 3 major moieties, the Functional Moiety (I), the Signaling Moiety (II) and Hydrophobic Moiety (III), and they differ on the number of methyl groups present on the chroman head.
The anticarcinogenic properties of vitamin E isomers and analogs and their synergistic effects in the presence of chemotherapeutic agents

It was originally thought that vitamin E’s chemopreventive properties could be attributed to its ability to neutralize the free radicals generated by the fecal bacteria in the gut and thereby prevent DNA damage. Nevertheless, accumulating evidence in the literature suggests that signal transduction activities independent of the antioxidant functions of vitamin E may also be responsible for the observed cancer chemopreventive effects.26,27 On the whole, and as it will be made clear in the following sections, evidence in the literature suggests that \( \alpha \)-TOC and \( \delta \)-TOC are more potent proapoptotic agents than \( \alpha \)-TOC, that tocotrienols are more effective proapoptotic agents than tocopherols (with \( \gamma \)-TT and \( \delta \)-TT being more potent than \( \alpha \)-TT and \( \beta \)-TT) and that the analogs \( \alpha \)-TOS and \( \alpha \)-tocopheryl ether-linked acetic acid (\( \alpha \)-TEA) are promising anticancer agents.

The antiproliferative and proapoptotic activities of tocopherols

In the past few years, several studies have been performed to investigate the specific antitumorigenic properties of tocopherols. As it will be shown in the rest of this section, an investigation of the specific properties of each tocopherol isomer has revealed that tocopherol isomers have different apoptotic potencies. In general, \( \gamma \)-TOC and \( \delta \)-TOC are more potent proapoptotic agents than \( \alpha \)-TOC despite the ability of the latter to induce cell cycle block. Since \( \alpha \)-TOC is the strongest antioxidant but incapable of inducing apoptosis, it seems that the antioxidant activity and apoptotic properties of vitamin E isomers are not coupled.

The different isoforms of vitamin E have distinct tissue distributions, but \( \alpha \)-TOC (Fig. 1) is the most abundant form of vitamin E in the plasma and tissue of the human organism as well as in vitamin supplements.25,28 Synthetic vitamin E is a chemical mixture composed of 12.5% authentic RRR \( \alpha \)-TOC and 87.5% of the 7 other \( \alpha \)-TOC stereoisomers (RRS-, RSR-, SSR-, SSS-, SRS-, SRR- and RSS).29 \( \alpha \)-TOC is known to be a free-radical-scavenging antioxidant important for protecting polyunsaturated fats from peroxidation.29 Nevertheless, despite the role of \( \alpha \)-TOC as an important antioxidant,3,30 accumulating evidence in the literature suggests that \( \alpha \)-TOC is not a strong proapoptotic inducer.27,31–34 For example, Campbell et al. (2006) reported that when SW480, HCT-15, HCT-116 and HT-29 colon cancer cells were treated with \( \alpha \)-TOC, no cell death was observed and this...
was contrary to the ability of \( \gamma \)-TOC to induce significant levels of apoptosis.\(^{27}\)

Despite the fact that there is no sufficient evidence in the literature to suggest that \( \alpha \)-TOC is a strong apoptotic inducer, data exists suggesting that this tocopherol plays a significant role in the inhibition of cell proliferation. For example, several studies have shown that a late G1 cell cycle block is observed when smooth muscle cells are treated with \( \alpha \)-TOC at concentrations in the range 10–50 \( \mu \)M, and the latter is believed to involve inhibition of protein kinase C.\(^{35,37}\) Even though these results propose a role for \( \alpha \)-TOC in the induction of cell cycle block, the more recent contradictory results that have been reported in both animal and human intervention studies\(^{38–40}\) suggest that \( \alpha \)-TOC is unlikely to be a good candidate for an adjuvant chemotherapeutic agent.

While extensive literature has been published on the potential health benefits of \( \alpha \)-TOC, little is known about \( \beta \)-TOC (Fig. 1). The role of \( \beta \)-TOC in the induction of apoptosis has not been investigated. Limited evidence in the literature, however, investigating the role of \( \beta \)-TOC on cell proliferation has shown that this compound does not cause inhibition of cell proliferation contrary to the antiproliferative effects of \( \alpha \)-TOC.\(^{35,37}\) These results therefore suggest that \( \beta \)-TOC is unlikely to block the cell cycle or induce apoptosis, but more detailed studies should be performed to investigate this matter in more detail.

\( \gamma \)-TOC represents the most abundant form of vitamin E in the diet in the USA but not in Europe (Fig. 1).\(^{41}\) In the American diet, \( \gamma \)-TOC is present at levels 2–4 times higher than that of \( \alpha \)-TOC.\(^{37}\) \( \gamma \)-TOC was ignored in the past due to its relatively low plasma and tissue concentration.\(^{42,43}\)

In a recent study by Ford et al. (2006), it has been estimated that the approximate mean serum concentration of \( \gamma \)-TOC in the US population is 5.74 \( \mu \)M compared to the much higher 30 \( \mu \)M concentration of \( \alpha \)-TOC.\(^{44}\) Interestingly, epidemiological evidence has shown that \( \gamma \)-TOC levels in plasma are associated with a lower risk for both colon and prostate cancer.\(^{45–47}\)

Recent evidence suggests that \( \gamma \)-TOC may be a more potent proapoptotic agent than \( \alpha \)-TOC.\(^{38}\) \( \gamma \)-TOC as well as the combination of \( \gamma \)-TOC with \( \delta \)-TOC induced apoptosis in androgen-sensitive prostate LNCaP cells but not in androgen-resistant PC3 cells as indicated by DNA fragmentation, annexin V staining, the induction of cytochrome c release, activation of caspase-9 and caspase-3 and cleavage of poly-ADP-ribose polymerase (PARP).\(^{39}\) Furthermore, treatment of colon cancer cells with varying characteristics (SW480, HCT-15, HCT-116 and HT-29) with \( \gamma \)-TOC resulted in significant cell death in all cell lines while treatment with \( \alpha \)-TOC did not. In addition, treatment with \( \gamma \)-TOC did not affect the normal cells. The induction of apoptosis in colon cancer cells by \( \gamma \)-TOC was confirmed with the cleavage of PARP and activation of caspase-3, caspase-7 and caspase-8 but not caspase-9.\(^{27}\)

In addition to its ability to induce apoptosis, \( \gamma \)-TOC has also been shown to be involved in the induction of cell cycle block. The arrest of the cell cycle progression in response to \( \gamma \)-TOC was shown to be accompanied by decreased levels of cyclin D1 and cyclin E.\(^{38}\) Galli et al. (2004) reported that in PC3 cells \( \gamma \)-TOC prevented proliferation at concentrations as low as 1 \( \mu \)M, whereas \( \alpha \)-TOC was much less effective with maximal inhibition of proliferation of less than 45% visible at a 50 \( \mu \)M concentration.\(^{51}\) In murine glioma C6 cells, \( \gamma \)-TOC induced cell cycle arrest at the GO/G1 phase of the cell cycle, which was associated with a lowered expression of cyclin E and cyclin-dependent kinases 2 and 4 as well as overexpression of p27.\(^{52}\) Furthermore, \( \gamma \)-TOC but not \( \alpha \)-TOC inhibited the proliferation of prostate LNCaP and PC3 and lung A549 cancer cells with cytotoxic effects on normal cells such as the PrEC normal prostate epithelial cells. The antiproliferative potency of \( \gamma \)-TOC is visible in the range of 10–50 \( \mu \)M and interestingly more pronounced inhibition of growth has been reported under conditions with relatively low serum (1–2% FBS) compared with higher serum concentrations (10% FBS).\(^{53}\) Since \( \gamma \)-TOC has a weaker antioxidant capacity than \( \alpha \)-TOC but inhibits cell proliferation as well as DNA synthesis more significantly than \( \alpha \)-TOC, it can be suggested that the \( \gamma \)-TOC-induced cell cycle arrest is independent of the antioxidant activity of the agent.\(^{50}\)

Similarly to \( \beta \)-TOC, not a lot of research has been performed to examine the proapoptotic activities of \( \delta \)-TOC (Fig. 1). Nevertheless, the limited evidence, which exists in the literature, supports a role for \( \delta \)-TOC in the induction of apoptosis. For example, \( \delta \)-TOC was shown to induce apoptosis in breast cancer MDA-MB-435 cells, whereas \( \beta \)-TOC and \( \gamma \)-TOC could not.\(^{53}\) Min et al. (2003) also showed that \( \delta \)-TOC was capable of inducing apoptosis in human hepatoma cells (HepG2) (at concentrations ranging from 12.5 to 200 \( \mu \)g/ml) and demonstrated that the order of apoptotic efficiency of the 3 vitamin E tocopherols was \( \delta \)-TOC > \( \gamma \)-TOC > \( \alpha \)-TOC.\(^{56}\) The difference in nature and magnitude of the anti-cancer effects of the 3 tocopherols did not correlate with their reported antioxidant activity. This suggests that it is the minor differences in the structure of tocopherols and not their antioxidant activity, which must be important for their biological proapoptotic activities. In addition, the combination of \( \gamma \)-TOC with \( \delta \)-TOC exhibited additive or synergistic induction of apoptosis in androgen-sensitive prostate LNCaP cells suggesting a role for both of these compounds in the apoptotic pathway.\(^{59}\) As with \( \gamma \)-TOC, tissue context seems to play an important role in the proapoptotic effects of \( \delta \)-TOC. Nevertheless, additional studies are necessary to further establish the role of \( \delta \)-TOC in the induction of apoptosis.

**The antiproliferative and proapoptotic activities of tocotrienols**

Although tocotrienols represent half of the natural vitamin E family, work on tocotrienols account for only ~1% of the total literature on vitamin E.\(^{55}\) This observation reveals that the properties and actions of tocotrienols have not been studied adequately and that future research should focus on identifying the specific activities of tocotrienols and comparing them to those of tocopherols.

The limited research performed on tocotrienols has revealed that this group of compounds possesses neuroprotective and cholesterol lowering but also proapoptotic properties not found in tocopherols.\(^{25,56–61}\) These findings are particularly interesting with regard to the role of these compounds as cancer chemotherapeutic agents since studies so far suggest that tocotrienols display a greater antitumor activity than tocopherols.\(^{57,56–62,63}\) without affecting normal cell growth and viability.\(^{56,64}\)

One of the first studies investigating the role of tocotrienols in neoplastic disorders reported that \( \alpha \)-TT and \( \gamma \)-TT were effective against sarcoma 180, Ehrlich carcinoma and invasive mammary carcinoma.\(^{65}\) In addition, when the effects of tocotrienols were directly compared to those of tocopherols in a rat mammary-tumor model, it was determined that only the latter enhanced tumor latency, an indicator of efficacy. Nevertheless, there was no effect of either compound on tumor multiplicity.\(^{66}\) Subsequently, studies reported that tocotrienols suppress the proliferation of a wide variety of tumor cells in culture including colon,\(^{57}\) breast\(^{57,58,60,69}\) and prostate.\(^{61,70}\)

The anticancer properties of tocotrienols were also evaluated in a variety of animal models. In these preclinical studies it has been shown that tocotrienols inhibit liver and lung carcinogenesis and suppress the growth of breast and melanoma tumors.\(^{65,66,67–71}\) In addition, tocotrienols display significantly more potent apoptotic activity in neoplastic mammary epithelial cells than tocopherols.\(^{57}\) Furthermore, tocotrienols are effective in inducing cell cycle arrest\(^{72}\) and activating p53,\(^{57}\) whereas the proapoptotic activities of tocotrienols seem to be related to their ability to inhibit Ras proteins and to modulate the ratio of Bax/Bcl-2.\(^{73,74}\)

When compared to all other tocotrienols, \( \alpha \)-TT seems to be the least potent proapoptotic inducer.\(^{77}\) However, in human Tenon’s fibroblasts, \( \alpha \)-TT inhibited cell proliferation with greater potency than \( \alpha \)-TOC and the 2 \( \alpha \)-TOC derivatives \( \alpha \)-tocopheryl ether-linked acetic acid (\( \alpha \)-TEA) and \( \alpha \)-tocopheryl succinate (\( \alpha \)-TOS).\(^{77}\) Furthermore, Sylvestre and Shah (2005) showed that treatment of neoplastic \( \alpha \)-SA mouse mammary epithelial cells
with α-TT in vitro resulted in a time-dependent, caspase-8- and caspase-3-mediated induction of apoptosis and that the latter was not associated with the activation of death receptors, but rather the inhibition of the PI3K/PDK/Akt mitogenic signalling pathway and downregulation in intracellular c-FLIP.69 Interestingly, 6-O-carboxypropyl-α-tocotrienol, a redox silent analog of α-TT, was cytotoxic against A349 cells, a human lung adenocarcinoma cell line.79 This observation suggests that the antioxidant or redox property of α-TT is not responsible for its anticancer property, further confirming the dissociation between antioxidant and apoptotic properties.

The apoptotic properties of γ-TT have been reported in highly malignant +SA mouse mammary epithelial cells. γ-TT causes induction of caspase-8 and caspase-3, but similarly to α-TT, the tocotrienol-induced caspase-8 activation is not associated with death receptor apoptotic signaling in these cells.61 γ-TT has also been shown to induce upregulation of Bax and fragments of Bid, activation of caspase-8, caspase-9 and caspase-3 and cleavage of PARP in human hepatoma Hep3B cells.71 A subsequent study has shown that the γ-TT induction of caspase-8 in malignant +SA mammary epithelial cells is associated with suppression in PI3K/PDK-1/Akt mitogenic signaling, inhibition of NF-κB activity and subsequent reduction in intracellular c-FLIP levels.80 More striking evidence for the possible role of γ-TT as an anticancer agent came from the study of Kumar et al. (2006).62 In this study, prostate tumors were induced by injecting PC3 cells into nude BALB/c mice, and it was shown that when the mice were subsequently injected with γ-TT (followed by irradiation), γ-TT reduced the size of the tumors by 40%.72

Despite the existence of only a limited number of studies, evidence suggests that δ-TT (Fig. 2) exerts more significant antiproliferative and antiangiogenic effects than α-TT, β-TT, and γ-TT.72 Gene expression analysis showed that δ-TT increased CYPIA1 gene, a phase I enzyme. These results agree with an earlier study which also showed that δ-TT was the most effective of the 3 tocotrienols tested (α-TT, γ-TT and δ-TT) in inducing apoptosis in both estrogen-responsive MCF-7 and nonresponsive MDA-MB-435 cells.73 In the breast cancer cell lines MCF-7 and MDA-MB-231, Shun et al. (2004) determined that δ-TT-induced apoptosis involves the upregulation and activation of the TGF-β receptor II and the TGF-β, Fas- and JNK-signaling pathways.19 In another study, Sylvester and Shah (2005) showed that treatment of neoplastic +SA mouse mammary epithelial cells with δ-TT resulted in a time-dependent increase in caspase-8 and caspase-3 activities (but no increase in caspase-9 activity).81 Interestingly, the combined treatment with specific caspase-8 or caspase-3 inhibitors completely blocked δ-TT-induced apoptosis, confirming the importance of these 2 caspases in the apoptotic process. In addition, in this study, it was shown that the activation of apoptosis in malignant (+)SA mammary epithelial cells was not mediated through the activation of death receptors, but appeared to result from the suppression of the PI3K/PDK/Akt mitogenic signalling pathway, and subsequent reduction in intracellular c-FLIP expression.69

In summary, evidence in the literature suggests that γ-TT and δ-TT are more potent proapoptotic agents than α-TT. The role of β-TT in apoptosis has not been determined because is present at very low levels in natural sources thereby making its extraction and use in research very difficult. Further studies of the actions and molecular mechanisms by which each tocotrienol induces apoptosis are necessary to evaluate the possible development as adjuvant chemotherapeutic agents.

**Improved proapoptotic activities of vitamin E synthetic analogs**

The 8 members of the vitamin E family are potent antioxidants since they all possess a free phenol hydroxyl group in their functional moiety (Moiety I, see Figs. 1 and 2). Nevertheless, as already discussed, these compounds vary in their ability to induce apoptosis. In the past few years, most research has focused towards structural variations within the functional moiety with the aim to develop the proapoptotic potency of these agents.29,81,82 The compounds developed in this manner were efficient against a variety of malignancies.7 The most studied member of these compounds is RRR-α-tocopheryl succinate (α-TOS), which has been shown to induce apoptosis in a variety of cancer cell lines, although at present several vitamin E derivatives are being studied. A more detailed explanation of the antiproliferative/proapoptotic properties of the compounds developed specifically as proapoptotic agents is provided below.

α-TOS has an ester-linked succinic acid moiety attached to the position-6 oxygen atom of the phenolic ring of the chroman head (Fig. 3). The succinyl moiety makes the analog redox silent such that the molecule can no longer act as an antioxidant.83,84 Previous studies have shown that the conversion of α-TOC to α-TOS greatly improves its anticancer action in tumorigenic cell lines and animal models.7,34,84–87 Since the apoptotic activity of the α-TOS molecule is much more improved than that of α-TOC and since the former lacks antioxidant activity, it can be concluded that the redox status of the molecule is not vital for its action as an antitu- morogenic agent. This notion is further supported by evidence provided throughout this communication.

Prasad and Edwards-Prasad (1982) were the first to suggest that α-TOS may be a useful therapeutic agent for tumors.85 Additional studies have demonstrated that α-TOS is a potent growth inhibitor of a wide variety of epithelial cancer cell types including prostate, breast, lung, colon, cervical and endometrial as well as hematopoietic-lymphoid leukemia, lymphoma and melanoma cells in vitro.82 It has also been shown that when α-TOS was administered to mammary epithelial cells with metastatic breast, lung, colon, cervical and endometrial as well as hematopoietic-lymphoid leukemia, lymphoma and melanoma cells in vitro,82 α-TOS was shown to have promising anticancer activity against the fatal malignant mesothelioma (when the latter was induced experimentally in immunocompromised mice)88 and the hard-to-treat HER2-positive breast cancer.96

α-TOS inhibits cell proliferation in MDA-MB-435 breast cancer cells by a GO/G1 cell-cycle block mediated in part by mitogen-activated kinases MEK1 and ERK1 and upregulation of the key cell cycle regulatory protein p21.81 In addition, cell cycle arrest was shown to be induced by α-TOS in osteosarcoma cell lines via activation of p53 and reduced expression of the transcription factor E2F1, which is known to be critical for the G1/S cell-cycle checkpoint.89 Induction of a G1/S cell cycle block was also visible in the androgen receptor (AR)-positive prostate cancer cell line LNCaP, where this effect was accomplished through downregulation of cell-cycle regulatory proteins cyclin D1, D3, E, cdk2 and 4, but not cdk6.90,91 α-TOS inhibits cell proliferation of prostate cancer cells at least in part by suppressing the expression of AR by means of transcriptional and posttranscriptional modulation, but not ligand binding, nuclear translocation or AR dimerization.93 Nevertheless, since this antiproliferative mode is specific for prostate cells, α-TOS must have several other tumor-suppressive activities. In fact, α-TOS at concentrations as low as 10 μg/ml (i.e. 25 μM) has been shown to restore the Fas (CD95) apoptotic signaling pathway in the human breast cancer cell lines MCF-7, MDA-MB-231, MDA-MB-435 and SKBR-3 via upregulation of the Fas receptor and ligand.100–102 Treatment of SGC-7901 gastric cancer cells with α-TOS also caused expression of Fas and the Fas-associated death domain (FADD) followed by caspase-8 activation.103 Furthermore, α-TOS has been shown to upregulate cell surface expression of TGF-β-type II receptor (TGF-β-RII) in MDA-MB-435 human breast cancer cells leading to increased cellular responsiveness to TGF-β-induced apoptosis.92,104,105 By restoring the TGF-β and Fas apoptotic signaling pathways, α-TOS contributes to the activation of c-Jun NH2-terminal kinase (JNK)-mediated apoptosis. Several reports have shown that α-TOS causes ROS and that the target of ROS must be the mitochondria, since the mitochondrially targeted coenzyme Q suppressed ROS accumulation and
The ability of α-TOS to activate apoptosis has also been attributed to its ability to downregulate the nuclear transcription factor NF-κB (Fig. 4). The latter is responsible for activating the inhibitors of apoptosis proteins (IAPs) and therefore by downregulating NF-κB, α-TOS can cause induction of apoptosis. α-TOS also has the ability of increasing the activity of protein kinases C-α (PKC-α). The inhibition of PKC-α prevents the phosphorylation of the antiapoptotic proteins Bcl-2 and Bcl-xL thereby allowing the translocation of Bax to the mitochondria. α-TOS is noteworthy not only for its induction of growth inhibitory effects on tumor cells but also by its lack of toxicity towards normal cells (e.g., intestinal epithelial, prostate and hepatocytes) and tissues.

One major disadvantage of α-TOS is that oral administration of this compound may not be effective due to the hydrolysis of the ester linkage by cellular esterases of the intestinal tract yielding α-tocopherol and succinic acid, neither of which exhibits anticancer properties. Another potential application of α-TOS was revealed by recent studies in allograft and xenograft models, where the compound is injected IP and therefore does not encounter the esterases. α-TOS has been found to be effective not only in suppressing lung, mammary and colon cancer in these animal models but also in preventing metastasis. These findings provide the exciting possibility that α-TOS may find application as a cancer therapeutic drug.
apoptosis capable of inducing human breast (MCF-7, MDA-MB-231, MDA-MB-435), ovarian (CP-70), cervical (ME-180), endometrial (RL-952), prostate (LNCaP, PC3, DU145), colon (HT-29, DLD-1), lung (A-549) and lymphoid (Raji, Ramos, Jurkat) cells to undergo apoptosis.\textsuperscript{29,87,156} Furthermore, \( \alpha\)-TEA is effective in reducing tumor burden and metastasis in syngeneic mouse mammary tumor models.\textsuperscript{84,123,127} Also like \( \alpha\)-TOS, \( \alpha\)-TEA does not induce apoptosis in normal human mammary epithelial cells, normal PrEC human prostate cells and tissues from healthy animals.\textsuperscript{29,84,87,123}

In a comparison of the \textit{in vitro} and \textit{in vivo} anticancer properties of \( \alpha\)-TOS and \( \alpha\)-TEA, Lawson et al. \textit{(2004)} showed that even though \textit{in vitro} both vitamin E derivatives were effective anticancer agents (inducing dose-dependent DNA arrest, inhibiting colony formation and inducing apoptosis), \textit{in vivo} when delivered by aerosol to BALB/c mice, \( \alpha\)-TEA was more effective as it reduced tumor burden and metastasis more effectively than \( \alpha\)-TOS.\textsuperscript{124} Because the acetic acid moiety attached to the phenolic ring of the chroman head of \( \alpha\)-TEA is linked by a nonhydrolyzable ether linkage, this molecule is more stable than \( \alpha\)-TOS. The latter may explain the better anticancer activities of \( \alpha\)-TEA reported in the previous studies.

The mechanism of \( \alpha\)-TEA-induced apoptosis has been deci- phered using cisplatin-sensitive (A2780S) and cisplatin-resistant (A2780/cp70R) human ovarian cancer cells.\textsuperscript{125} \( \alpha\)-TEA treatment at 20 \( \mu \)M for A2780S cells, and at 40 \( \mu \)M for A2780/cp70R cells, caused increased levels of membrane-associated Fas, activation of JNK, downregulation of Akt, Erk1/2, c-FLIP and Survivin, conformational change of Bax, cleavage of Bid and activation of caspase-3, caspase-8 and caspase-9.\textsuperscript{128} These data suggest that \( \alpha\)-TEA is a potent proapoptotic inducer, which activates both the extrinsic apoptotic pathway (revealed by upregulation of the Fas receptor) as well as the intrinsic pathway (shown by activation of caspase-9), but also suppresses antiapoptotic Akt and Erk targets. In this respect, \( \alpha\)-TEA is a very potent apoptotic inducer having the added advantage of being resistant to hydrolysis by cellular esterases.

Despite the well known proapoptotic properties of \( \alpha\)-TOS, this compound has poor solubility, limiting its therapeutic efficacy. The conjugation of \( \alpha\)-TOS to polyethylene glycol succinate (PEG) results in the production of a compound known as \( \alpha\)-tocopheryl polyethylene glycol sucinate (TPGS) (Fig. 3b). The latter is an amphiphilic compound, and due to its good solubility in water, it has been used as a vehicle for drug delivery systems and to enhance the bioavailability of poorly absorbed drugs.\textsuperscript{129,130}

To evaluate the potential proapoptotic properties of TPGS, Youk et al. \textit{(2005)} used both a cell culture system and the nude mouse xenograft model transplanted with human lung carcinoma cells.\textsuperscript{131} TPGS was more effective than \( \alpha\)-TOS in inducing apoptosis in the human H460 and A549 cancer cells as well as in the \textit{in vivo} model. Paradoxically, the strongest proapoptotic effects of TPGS were not due to increased uptake of the compound but rather to the ability of the compound to generate ROS, which is known to trigger the mitochondrial pathway of apoptosis.\textsuperscript{131} Nevertheless, further studies need to be performed to investigate the exact molecular mechanism by which TPGS causes induction of apoptosis in the cell.

There is very limited evidence regarding the proapoptotic activities of \( \beta\)-tocopheryl, \( \gamma\)-tocopheryl and \( \delta\)-tocopheryl succinates (\( \beta\)-TOS, \( \gamma\)-TOS and \( \delta\)-TOS) (Fig. 3a). Burringer et al. \textit{(2003)} compared the proapoptotic activities of \( \beta\)-TOS, \( \gamma\)-TOS and \( \delta\)-TOS in the human T lymphoma cell line Jurkat, the neuroblastoma cell line HTB11, the breast carcinoma cell line MCF7 and its caspase-3-expressing variant.\textsuperscript{132} They found that \( \alpha\)-TOS possesses the highest apoptotic activity followed by \( \beta\)-TOS, \( \gamma\)-TOS and \( \delta\)-TOS. Replacement of the succinyl group with a maleyl group greatly enhanced the activity, while it was lower for the glutaryl esters. Furthermore, methylation of the free succinyl carboxyl group on \( \alpha\)-TOS and \( \delta\)-TOS completely prevented the apoptotic activity of the parent compounds, whereas both Trolox and its succinylated derivative were inactive. \( \gamma\)-TOS (Fig. 3a) was found to induce higher levels of apoptosis than \( \gamma\)-TT when tested in a number of tumor cell lines, whereas the conversion of \( \gamma\)-TT to \( \gamma\)-TS (\( \gamma\)-tocotrienol succinate) improved its apoptotic potency.\textsuperscript{132}

To find analogs with higher apoptogenic efficacy, Tomic-Vatic et al. \textit{(2005)} prepared novel compounds where the ester bond was replaced by an amide bond.\textsuperscript{133} All of these analogs were significantly proapoptotic than their ester counterparts, with \( \alpha\)-tocopheryl maleyl amide being the most effective. Importantly, methylation of the free carboxylic group completely eradicated the apoptogenic activity of the compounds. Similarly, as shown for the ester amides, the amides induced apoptosis by mitochondrial destabilization. The superiority of amides over the ester analogs was suggested to be due to their higher partitioning into the lipid phase.\textsuperscript{134}

\section*{Synergistic antitumorigenic effects of vitamin E derivatives in the presence of chemotherapeutic agents}

In an attempt to investigate the possibility of synergistic anticancerogenic effects between chemotherapeutic agents and vitamin E, Peralta et al. \textit{(2006)}, examined the proliferation of estrogen receptor positive breast cancer cell lines MCF-7 and T47D following treatment with tamoxifen in the presence or absence of \( \alpha\)-TOC.\textsuperscript{135} The results of this study showed that \( \alpha\)-TOC decreased the inhibitory effect of tamoxifen and eliminated the rapid increase in intracellular calcium that leads to tamoxifen-stimulated apoptosis.

Despite these discouraging results, a number of studies have supported the possible use of vitamin E with chemotherapeutic agents. In a study by Weber et al. \textit{(2002)}, it has been shown that \( \alpha\)-TOS increases the levels of apoptosis produced by TRAIL both \textit{in vitro} and in experimental colon cancer.\textsuperscript{33} In fact, \( \alpha\)-TOS has been shown to significantly decrease the high IC\textsubscript{50} values for TRAIL by a factor of \textasciitilde 100.\textsuperscript{135} Dalen and Neuzil \textit{(2003)} suggested that the increased apoptosis in the presence of the 2 compounds is due to the inhibition of the NF-\kappaB pathway, which is activated by TRAIL.\textsuperscript{112} Studies have shown that the induction of apoptosis in the presence of \( \alpha\)-TOS and TRAIL is caspase- and p53-dependent and involves the upregulation of receptors DR4 and DR5.\textsuperscript{109,135} In addition to the increased apoptotic effects with the combined treatment of cells with \( \alpha\)-TOS and TRAIL, \( \alpha\)-TOS increases the growth inhibitory effects of several other chemotherapeutic agents such as those of cisplatin, tamoxifen and decaprost in melanoma cells,\textsuperscript{136} parotid acinar carcinoma cells,\textsuperscript{137} as well as those of Adriamycin on prostate carcinoma cells,\textsuperscript{138} and those of doxorubicin on leukemia cells.\textsuperscript{139} Dietary \( \alpha\)-TOS, selenium and lycopene also had a synergistic inhibitory effect on prostate cancer incidence in transgenic mice, further supporting the notion that \( \alpha\)-TOS may have anticarcinogenic properties, which should be further confirmed in clinical studies.\textsuperscript{140} These results, therefore, suggest that \( \alpha\)-TOS may be an anticancer agent and/or adjuvant of considerable therapeutic potential.

The combination of \( \alpha\)-TEA with celecoxib caused a decrease in breast tumor volume in nude mice, whereas the volume of lung metastases in the \( \alpha\)-TEA and celecoxib group was significantly lower than for either separate treatment.\textsuperscript{141} Similarly, combinations of \( \alpha\)-TEA and cisplatin significantly reduced tumor burden and metastases in a xenograft model of cisplatin-resistant human ovarian cancer cells.\textsuperscript{142} These reports provide a great promise for the combination \( \alpha\)-TEA and certain chemotherapeutic agents in the treatment of ovarian and breast cancer.

\section*{The role of mitochondria in the induction of apoptosis by vitamin E analogs}

The vitamin E analogs belong to the family of “mitocans” \textit{i.e.}, agents that initiate programmed cell death by targeting the mitochondria of tumor cells.\textsuperscript{7,76,143} While for many years the initial events involved in the induction of apoptosis by vitamin E analogs had not been resolved, recent studies have identified the initial
target for vitamin E analogs. These studies showed that α-TOS targets complex II of the respiratory chain to displace ubiquinone binding. It is therefore possible that disrupting the electron flow of mitochondrial complex II results in generation of ROS in the form of superoxide, triggering mitochondrial destabilization and initiation of apoptotic pathways.

While still a lot of more research needs to be performed to understand even further the initial events that take place during the induction of apoptosis, the subsequent events are known in much more detail. In summary, proapoptotic proteins Bax and Bak translocate to the mitochondrial outer membrane causing its destabilization and the release of cytochrome c into the cytosol where it forms a complex with Apaf-1 and procaspase-9 (Fig. 4). The latter gets activated into caspase-9 by autocatalytic cleavage and subsequently activates the effector caspase-3, caspase-6 or caspase-7. At this stage, the cell enters the “commitment” phase of apoptosis (Fig. 4). Strong evidence supporting the essential role of mitochondria in the induction of apoptosis by vitamin E analogs came from experiments with mtDNA-deficient (p<sub>5</sub>) cells, which were found to be resistant to α-TOS when compared to their wild-type and revertant counterparts. It has also been observed that transfection of cancer cells with dominant-negative caspase-9, or caspase-8 siRNA, suppressed apoptosis induced by α-TOS, suggesting that caspase-9 is vital for the induction of apoptosis by the vitamin E analog. The mitochondrial proapoptotic and antiapoptotic proteins such as Bax and Bcl-2, respectively, are also known to be important in the induction of apoptosis. The ratio of pro- to antiapoptotic proteins is known to be important in the production of the mitochondrial permeability transition pore. The significance of these mitochondrial proteins in the induction of apoptosis by vitamin E is well established. For example, Neuzil et al. (2006) have reported Bax mitochondrial relocalization in cancer cells exposed to α-TOS. Furthermore, other studies showed that overexpression of Bcl-2 or Bcl-xL protected, whereas overexpression of Bax sensitized, cells to α-TOS-induced apoptosis. As expected, downregulation of Bcl-2 by antisense oligodeoxynucleotide treatment sensitized cells to the vitamin E derivative. In addition to causing the relocalization of proapoptotic proteins to the mitochondria, a more recent study by Shiah et al. (2006) has shown that α-TOS induces apoptosis by also binding to the BH3 domain of antiapoptotic proteins and therefore promotes cell survival. Furthermore, active Akt causes the activation of the transcription factor NF-κB. The latter (composed of p50 and p65 subunits) is commonly present in the cytoplasm in an inactive form (by being bound to the inhibitor IκBα). Upon activation, there is phosphorylation and ubiquitination of IκBα, which is subsequently degraded. Free NF-κB then translocates from the cytoplasm to the nucleus where it leads to the transcription of several genes including those coding for inhibitors of apoptosis IAPs and the caspase inhibitor c-FLIP.

Studies have shown that γ-TT, γ-TT and δ-TT induce caspase-8- and caspase-3-mediated apoptosis not via the classical activation of death receptors, but rather via the inhibition of the PI3K/PDK/Akt mitogenic signalling pathway and the downregulation in intracellular c-FLIP. A recent study has shown that γ-TT decreased the intracellular levels of activated PI3K and reduced phospho-Akt kinase activity in neoplastic +SA mammary epithelial cells. A more recent study by Samant and Sylvester (2006) showed that γ-TT treatment in the same cells caused a significant decrease in the ErbB3 tyrosine phosphorylation. Because ErbB1 or ErbB2 receptors form heterodimers with the ErbB3 receptor, and ErbB3 heterodimers have been shown to be the most potent proapoptotic activators of PI3K, it is suggested that the antiproliferative effects of γ-TT in neoplastic +SA mouse mammary epithelial cells are mediated by a suppression in ErbB3-receptor tyrosine phosphorylation with subsequent reduction in PI3K/PDK-1/Akt mitogenic signaling. The decreased activity of Akt must prevent the phosphorylation of caspase 9 and Bad, thereby causing the activation of these proteins and allowing their participation in the apoptotic process.

In addition to downregulating the prosurvival pathway PI3K/PDK/Akt, Ah et al. (2007) showed that γ-TT suppresses the TNFα-induced NF-κB activation as well as NF-κB activation induced by carcinogens, growth factors and inflammatory stimuli. These observations are consistent with previous reports from Shah and Sylvester (2005) showing that the mode of inhibition of NF-κB by γ-TT involves the inhibition of IKK activation, IκBα phosphorylation and IκBα degradation. The inhibition of IKK β by γ-TT must be at least in part the result of decreased Akt activity. As already mentioned, NF-κB is responsible for activating the IAPs including c-IAP1, c-IAP2 and x-IAP. Consequently, γ-TT seems to induce apoptosis by inhibiting the expression of NF-κB-dependent gene products. Therefore, γ-TT downregulates the levels of antiapoptotic proteins (Bcl-xL, Bcl-2, IAP, XIAP, Bfl-1/A1, TRAF1, Survivin, and cFLIP), thereby allowing the induction of apoptosis. Additionally, the inactivation of NF-κB by γ-TT also leads to the downregulation of genes involved in proliferation (cyclin D1, COX2 and c-Myc), invasion (MMP-9 and ICAM-1) and angiogenesis (VEGF).

Similar to γ-TT, α-TOS has been shown to cause induction of apoptosis by downregulating NF-κB. α-TOS suppresses NF-κB at least in part via activation of caspase-3 that cleaves the NF-κB subunit p52, thereby inactivating NF-κB. Interestingly, in a more recent study, Crisp et al. (2007) have shown that the downregulation of the activity of NF-κB by α-TOS leads to the downregulation of antiapoptotic proteins (x-IAP and Bcl-xL) in androgen-dependent LNCaP cells and also causes decreased expression of several proangiogenic and prometastatic proteins (IL-6, IL-8, VEGF, ICAM-1) in androgen-independent PC3, DU145 and CA-HPV-10 cells. These results therefore suggest that vitamin E analogs may prove to be useful compounds for the prevention or treatment of cancer metastasis.

In addition to the involvement of the NF-κB pathway in the induction of apoptosis by α-TOS, the extracellular signal-regulated kinases (ERKs) and the c-Jun NH2-terminal kinase (JNK), but not the p38MAPK pathway, have been implicated in the induction of apoptosis by vitamin E analogs. For example, ERK1/2 and JNK have been shown to be transiently activated and involved in the induction of apoptosis in the human breast cancer MDA-MB-435 cells by α-TOS. In this study, the ERKs and
JNK have been shown to subsequently cause the activation of the nuclear transcription factors c-Jun and ATF2. Even though the upstream activators of MEK1 that are activating the ERKs and are playing a role in α-TOS-induced apoptosis remain to be identified, it is currently believed that Fas may be activating JNK via Daxx and that TGF-β may be causing this activation via Rho/CDK12 and TAB/TAK-1 signaling.32 The activation of JNK by α-TOS has also been reported in prostate cancer cells154 and in gastric cancer cells.355 The induction of apoptosis by the vitamin E analog α-TEA also involves the activation of JNK and the downregulation of Akt, Erk1/2 and c-FLIP.128 In a recent study, it has been shown that α-TEA-triggered apoptosis causes dual signaling from Fas with essential roles for both FADD and Daxx, with FADD initiating the classical pathway mediated by caspase-8 activation and Daxx initiating the alternate pathway involving activation of JNK, c-Jun and increased levels of Fas and Fasl.156 Overall, these results suggest that the above signaling pathways are key players in the vitamin E analog-induced apoptotic response.

Selectivity of vitamin E analogs for cancer cells

One of the major advantages in using vitamin E analogs as chemotherapeutic agents is their selectivity for cancer cells. This selectivity may depend on a lot of factors. Neuzil et al. (2007) have proposed that α-TOS is selective for cancer cells at least in part due to the reduced antioxidant defenses expressed by these malignant cells when compared to their normal (nonmalignant) counterparts.76 For example, as mentioned previously, the exposure of cancer cells to α-TOS causes rapid accumulation of ROS, which is a prerequisite for apoptosis. This is caused by the fact that cancer cells commonly express lower levels of antioxidant enzymes, such as the manganese superoxide dismutase (MnSOD), than normal cells.157 On the other hand, the higher levels of antioxidant enzymes in normal cells prevent the accumulation of ROS and therefore the induction of apoptosis in normal cells. Another reason why α-TOS is selective for cancer cells may be due to the lower esterase activity expressed by these malignant cells when compared to their normal (nonmalignant) counterparts. Therefore, the higher esterase activity in normal cells may result in the hydrolysis of α-TOS yielding α-TOC and succinic acid, neither of which exhibits anticaner properties.76,84 More importantly, the selectivity of vitamin E analogs for cancer cells may be dependent on the ability of the former to specifically target and downregulate certain prosurvival pathways such as the PI3K/Akt and the NF-κB pathways, which are abnormally upregulated in carcinogenic but not normal cells.32,114,128,148

Vitamin E and caspase-independent apoptosis: Emergence of a novel apoptotic pathway?

Although a number of studies support that vitamin E-induced cell death is caspase-dependent, there are other reports supporting the caspase-independent induction of cell death. For example, the irreversible pancaspase inhibitor z-VAD-fmk provides only a minor protection from the apoptotic effects of γ-TOC in prostate cancer LNCaP cells suggesting that both caspase-dependent and -independent mechanisms are involved in the induction of apoptosis by γ-TOC in these cells.158 Incubation of MDA-MB-231 human breast cancer cells with γ-TT produced morphological changes associated with classical apoptosis including membrane blebbing, chromatin condensation and fragmentation, whereas the annexin V-binding assay detected the translocation of membrane phospholipid. This was followed by collapse of the mitochondrial membrane potential and cytochrome c release from mitochondria. Nevertheless, the expression of Bax and Bcl-2 (mRNA and protein) did not change and cleavage of poly-(ADP-ribos)e-polymerase was not detected. These results suggest that caspases were not involved in γ-TT-induced apoptosis in MDA-MB-231 cells.159 However, apoptosis induced by the same agent in +SA cells was not associated with disruption or loss of mitochondrial membrane potential, or the release of mitochondrial cytochrome c into the cytoplasm.151 Interestingly, apoptotic +SA cells showed a paradoxical decrease in mitochondrial levels of proapoptotic proteins Bid, Bak and Bad, and a corresponding increase in mitochondrial levels of antiapoptotic proteins, Bcl-2 and Bcl-xL, suggesting that mitochondrial membrane stability and integrity might actually be enhanced for a limited period of time following exposure to γ-TT.151 A possible explanation for the caspase-insensitive events is that apoptosis may be partly regulated by lysosomes.160 In a study by Neuzil et al. (2002), it has been shown that cells deficient in the lysosomal protein cathepsin D were relatively resistant to α-TOS.161 Thus, even though mitochondria seem to be essential for the initiation of apoptotic signaling by some vitamin E derivatives, other analogs may exert lysosomal stabilization. This is particularly significant to compounds like γ-TT and δ-δTT where mitochondrial-independent events have been reported.72,162

The previous reports suggest that the cellular microenvironment may determine not only the induction of apoptosis, but it may also control the apoptotic pathways. An example where the actual cell type seems to be regulating whether apoptosis takes place or not was reported in LNCaP prostate cancer cells, where activation of caspase-9 was evident following exposure to γ-TOC.79 while in colon cancer cell lines (SW480, HCT-15, HCT-116 and HT-29), there was neither caspase-9 activation nor apoptosis induced by γ-TOC.27 With respect to the selection of apoptotic pathway, an example comes from γ-TT, which was found to cause induction of caspase-8 and caspase-3 but not caspase-9 in highly malignant +SA mouse mammary epithelial cells,61 but caused activation of caspase-8, caspase-3 and caspase-9 in human hepatoma Hep3B cells.77 These observations suggest that molecular differences (cellular context) in the various cell types may influence the apoptotic pathway induced by each vitamin E isomer. This suggestion is further supported by the observation that different percentages of apoptosis are visible when carcinogenic cell lines of the same tissue origin (i.e. colon) are treated with γ-TOC.25

Conclusion

The redox-active vitamin E isomer α-TOC is incapable of inducing apoptosis in tumor cell lines and may not be a suitable chemotherapeutic agent.71,31–34 Nevertheless, its antioxidant properties may still be useful in the chemoprevention of cancer. This suggestion is supported by the observations derived from several epidemiological studies indicating a correlation between consumption of the redox-active α-TOC and lower levels of colon, rectal and prostate cancer.17,163 Nevertheless, analysis of the evidence presented in the literature regarding the antitumorigenic activities of the different vitamin E isomers suggests that even though α-TOC is incapable of inducing apoptosis, γ-TT and δ-δTT induce apoptosis in a variety of cancer cell lines.72,61,77,80 In addition, some vitamin E analogs and especially α-TOS have also been shown to have antitumorigenic properties. The selectivity of these vitamin E analogs in inducing apoptosis in cancer cells without affecting normal cells provides additional support for their usefulness as cancer chemotherapeutic agents.4 While individual analogs of vitamin E may differ, functionally they often significantly overlap by targeting the mitochondria and inducing apoptosis in the cell.144

The combined evidence from the literature reviewed in this paper suggests that the synthetic compounds are superior proapoptotic inducers than the naturally occurring compounds. Therefore, the proapoptotic activities of all of the newly synthesized derivatives should be evaluated in detail as these may present different antitumorigenic potencies. Since there seems to be no direct link between the antioxidant activity of each isomer/derivative and its proapoptotic potency, structural variations within the Functional Moiety of vitamin E may be the most important in determining their proapoptotic potent. Even though α-TOS is considered a promising chemotherapeutic agent, further vitamin E
derivatives could be designed and tested initially in in vitro systems. These compounds should be designed with the main scope to overcome the hydrolysable property of α-TOS, which is considered to be its main disadvantage due to the presence of cellular esterases in the intestinal tract, which are likely to convert α-TOS to the nonfunctional α-TOC and succinic acid molecule.82,84,90,118,120 The design of nonhydrolyzable vitamin E derivatives, such as α-TEA, has been very promising.84,123,125 Our own experience in evaluating the proapoptotic activities of nonhydrolyzable derivatives of vitamin E has been very positive (unpublished data).

In addition to overcoming the hydrolysable disadvantage of α-TOS, new derivatives could be based on structural variations of the effective tocotrienols γ-TT and δ-TT. These tocotrienols are more effective than α-TOC and therefore changes in the functional domain of these compounds could produce derivatives with much greater proapoptotic potency than α-TOS. Despite the lack of sufficient studies in the literature, the few studies investigating, e.g. the conversion of γ-TT to γ-TS, have shown that the addition of succinate to γ-TT produces a compound with an even much greater proapoptotic potency.122 It is therefore possible that changes in the functional domain of the tocotrienols and particularly their conversion to nonhydrolyzable derivatives could produce even more potent anticarcinogenic derivatives. Therefore, the undertaking of additional in vitro studies to examine the proapoptotic potencies of these derivatives would be vital to select the most promising synthetic derivatives that could then be tested in in vivo animal studies. Of course, it will be very important to establish the role of each vitamin E analog in each type of cancer, since the suitability of each compound may depend on the type of cancer and the tissue context. Successful results from animal studies could then justify the testing of these compounds in clinical studies.

In addition to the designing and testing of novel derivatives of vitamin E, further research should focus on examining the possible synergistic effects that could be obtained by the use of combinations of vitamin E isomers or derivatives in the presence of other anticancer agents. As shown in this review, the results obtained so far from the use of vitamin E analogs (e.g., α-TOS and α-TEA) and several chemotherapeutic agents are encouraging. Nevertheless, this area of research should be more actively pursued in animal models. It is becoming apparent from this review that the time is ripe for testing the most effective tocotrienols γ-TT and δ-TT as well as other nonhydrolyzable derivatives of vitamin E in animal models of cancer therapeutics. A possible synergy between a vitamin E isomer or derivative and one or more chemotherapeutic agents could be very significant, since it would suggest that a desired biological response could be achieved by a lower concentration of the chemotherapeutic agent. Lower concentrations of chemotherapy could in turn translate into lower cell toxicity, with consequent beneficial effects for patients with cancer.

As it has already been presented in this review article, the mitochondria seem to be the main regulators in the induction of apoptosis.7,76 The fact that vitamin E compounds target the mitochondria provides a theoretical basis to hypothesize that these agents may remain effective following DNA mutations that occur in cancers, making them superior to many commonly used drugs whose mode of action relies mainly on the activation of the tumor sup-

**FIGURE 5** – A model for the treatment of cancer in the presence of vitamin E analogs. Usually, cancer is treated with the use of chemotherapeutic agents that are dependent on the activation of caspases commonly via activation of the tumor suppressor protein p53. Nevertheless, tumors commonly develop resistance to this pathway with adverse effects for patient outcome. Therefore, a possible treatment with a standard chemotherapeutic agent that induces caspase-mediated apoptosis in the presence of a vitamin E analog that activates both caspase-dependent and caspase-independent models of cell death could probably lead to a more successful eradication of cancer and lower levels of tumor resistance.
pressor 53,76 Furthermore, the ability of vitamin E isomers and derivatives to induce caspase-independent apopto-
sis77,72,151,158,160,162 provides another advantage for their possible use in tumor therapy, since these compounds may prove to be useful in the treatment of tumors that are resistant to therapeutic agents that are dependent on the activation of caspases. Therefore, a potential treatment with a standard chemotherapeutic agent that induces caspase-mediated apoptosis in the presence of a vitamin E analog that activates a caspase-independent mode of cell death could probably lead to a more successful eradication of cancer and lower levels of tumor resistance (Fig. 5). A very important aspect of vitamin E analogs is their targeting of the main survival pathways of the cell (i.e., PI3K and NF-κB), which provides not only an amplification response of the apoptotic pathway, but also kills selectively cancer cells whose survival may depend on activating these pathways.12,113,118,153

Overall, the reports presented in this review suggest that vitamin E analogs provide great promise in the treatment of cancer. Therefore, what remains to be seen in the future is the actual testing of these novel agents in the preclinical and clinical setting.

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751 ANTICANCER ACTIVITIES OF VITAMIN E ISOMERS AND ANALOGS


