Review Article

Theme: Transporters and Cancer Therapy

Guest Editor: Rajgopal Govindarajan

Vitamin E Transporters in Cancer Therapy

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Received 2 September 2014; accepted 17 November 2014; published online 3 December 2014

Abstract. Besides their potent antioxidant activity, vitamin E isoforms demonstrated multiple therapeutic activities among which is their activity against different cancer types, including breast, prostate, and colon cancers. However, the activity of vitamin E isoforms is limited by their low bioavailability following oral administration. In addition to the low solubility, vitamin E isoforms have been established as substrates for several intestinal and hepatic transport proteins. In this review, we present reported anticancer activity of vitamin E family members and the possible utilization of vitamin E and derivatives as chemosensitizers to reverse multidrug resistance when given as part of a delivery system and/or in combination with anticancer therapeutic drugs. Then, the review discusses disposition of vitamin E members and transport proteins that play a role in determining their systemic bioavailability followed by recent advances in vitamin E formulations and delivery strategies.

KEY WORDS: Bioavailability; Cancer therapy; Transporters; Vitamin E.

INTRODUCTION

Vitamin E is the term given to a group of natural compounds first discovered in 1922 by Evans and Bishop (1). These natural hydrophobic fat-soluble compounds are found in a variety of food sources and have wide range of biological activities that are essential for normal physiology (2). The basic structure of vitamin E contains a polar chromanol head group with a long isoprenoid side chain. There are eight naturally occurring forms of vitamin E, namely, alpha (α), beta (β), gamma (γ), and delta (δ) isoforms of tocopherol and tocotrienol. The structural difference between both groups of isoforms is that tocopherols have a saturated phytyl chain while tocotrienols have the unsaturated chain (Fig. 1). The small structural differences between vitamin E isoforms have a significant impact on their biological activities and fate inside the body. Among these isoforms, α - and γ -tocopherols present in major amounts with relative proportions, depending on the source (3). Naturally occurring α -tocopherol exists in the RRR stereo-isoform, while its synthetic process yields all-rac α -tocopherol that exists in equal amounts of eight different stereo-isomers (4). According to the dietary guide published by the United States Department of Agriculture (USDA), the dietary recommended intake for vitamin E is 15 mg for both males and females aged 14 and over, and the tolerable upper intake level is reported as 1000 mg in people aged 19 and over (5). The mean daily intake of α -, β -, γ -, and

δ -tocopherol and α -, β -, γ -, and δ -tocotrienol in humans has been reported as 8.76, 0.69, 7.58, 1.56, 1.44, 2.51, 0.20, and 0.06 mg, respectively (6).

Vitamin E exhibits various functions including antioxidant, anti-inflammatory, and anti-thrombolytic activities, in addition to other therapeutic activities (7,8). Differences between tocopherols and tocotrienols including their therapeutic activities are summarized in Table I. Protection of lipids and membranes from oxidative damage by quenching free radicals or peroxides is considered the main function of vitamin E isoforms with powerful antioxidant activity (9). Reactive oxygen species (ROS) such as hydroxyl and peroxy radicals, produced and released primarily by the mitochondria during lipid peroxidation, could alter the cell membrane and lead to modified proteins and DNA bases (10,11). Vitamin E isoforms are able to inhibit lipid peroxidation in varying degrees with α -tocopherol showing the highest activity among tocopherols in the following order: α -tocopherol > β -tocopherol > γ -tocopherol > δ -tocopherol (12). On the other hand, compared with tocopherols, tocotrienols have demonstrated a stronger antioxidant activity in several studies using *in vivo* and *in vitro* models (13). Individual tocotrienols displayed different antioxidant potencies in the following order: δ -tocotrienol > γ -tocotrienol > β -tocotrienol > α -tocotrienol (14,15).

In recent years, vitamin E isoforms and most importantly tocotrienols have generated much interest as they have been reported to possess anticancer and tumor-suppressing activities. However, one of the major issues associated with vitamin E isoform utilization as anticancer drugs is their limited systemic availabilities. This review highlights and summarizes recent reports on the anticancer activity of vitamin E isoforms and their potential to serve as therapeutic

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Fig. 1. Chemical structures of vitamin E isoforms

drugs for treatment of different cancers. In addition to their anticancer effect, this review discusses their activity as chemosensitizers to enhance the anticancer activity of different chemotherapy agents. The review also describes the disposition of vitamin E isoforms and role of transport proteins in determining their blood levels and thus therapeutic activities, and finally introduces available strategies to overcome vitamin E low oral bioavailability.

VITAMIN E ISOFORMS AS POTENTIAL ANTICANCER AGENTS

In Vitro and *In Vivo* Studies

The therapeutic potential of vitamin E isoforms in cancer therapy has been widely studied and thoroughly reviewed (16–18). Vitamin E members could be beneficial against a variety of cancer types, including breast, prostate, and colon through various possible mechanisms, including stimulation of wild-type p53 tumor suppressor gene, down-regulation of mutant p53 proteins, activation of heat shock proteins (HSPs), and possession an anti-angiogenic effect mediated by the blockage of transforming growth factor (TGF) (18–23). The anticancer activity of vitamin E isoforms have been evinced by several

in vitro and *in vivo* studies. Available reports have shown α-tocopherol to inhibit cancer cell growth through the suppression of PKC and collagenase production (24). Other studies have established that γ-tocopherol as more effective than α-tocopherol in its growth inhibitory effect on human prostate cancer cell lines and in a transgenic rat for adenocarcinoma of prostate (TRAP) model (24,25). Furthermore, *in vitro* studies in the human prostate cancer cell line DU-145 demonstrated the inhibition effect of γ-tocopherol on cell cycle progression via reduction of cyclin D1 and cyclin E levels (24).

Besides, the natural isoform α-tocopherol is also commercially available as synthetic esters. Among the synthetic derivatives are α-tocopheryl acetate, α-tocopheryl succinate, and α-tocopheryl polyethylene glycol succinate (TPGS), which demonstrated greater antioxidant activity compared with the natural isoform. Numerous studies have indicated that the synthetic tocopheryl succinate (TS) has the most effective anticancer activity among the natural and synthetic vitamin E family members. TS has shown to be highly selective against different malignant cells, including breast, prostate, lung, stomach, ovary, and colon cancer cells but largely safe to normal cells (17). TS inhibits cancer cell growth by several mechanisms, including (i) inhibition of cell proliferation by blocking G0/G1 cell-cycle mediated in part

Table I. A Summary of the Differences Between Tocopherols and Tocotrienols

	Tocopherols	Tocotrienols	Ref
Structure	Contain a polar chromanol head group with a long saturated phytol chain	Contain a polar chromanol head group with a long unsaturated phytol chain	(14)
Antioxidant activity	Exert strong antioxidant activity	Exert strong antioxidant activity	(11,12,90)
Anti-inflammatory effect	Provide minimal anti-inflammatory effect	Provide strong anti-inflammatory effect	(91,92)
Neuroprotective effect	Have no role as neuroprotective agents	Have strong neuroprotective effect	(93–95)
Lipid-lowering activity	Do not have lipid-lowering activity	Have relative moderate effect	(96,97)
Anticancer effect	Weak to none	Exert anticancer activity	(17,24,43,47)

by mitogen-activated kinases MEK1 and ERK1, and upregulation of the cell cycle regulatory protein p21 (26), (ii) induction of apoptosis that is attributed to TS ability to down-regulate the nuclear transcription factor NF- κ B (27), and (iii) inhibition of metastasis (28). Findings from the *in vitro* studies were confirmed *in vivo*, however only following the intraperitoneal administration of TS which was effective in reducing the incidence of breast, colon, and stomach cancers and melanoma, whereas when given orally TS was ineffective due to extensive hydrolyses by esterases in the gastrointestinal tract (29). Furthermore, a nonhydrolyzable RRR- α -tocopherol ether-linked acetic acid analogue (α -TEA) exhibited *in vitro* and *in vivo* antitumor activity using a syngeneic BALB/c mouse mammary tumor model and lung metastases were significantly reduced (30).

Tocotrienols are known to possess potent anticancer activity with γ - and δ -tocotrienols being the most potent isoforms of all naturally existing tocotrienols (31). Several *in vitro* and *in vivo* studies have investigated the anticancer activity of tocotrienols in different cancer types. Oral administration of tocotrienols resulted in a significant suppression of liver and lung carcinogenesis in mice (31). In the human hepatocellular carcinoma HepG2 cells, δ -tocotrienol exerted more significant antiproliferative effect than other tocotrienol isoforms (32). Treatment with tocotrienol-rich fraction (TRF) caused a G0/G1 phase arrest and sub-G1 accumulation in three different human prostate cancer cell lines (33). In addition, γ -tocotrienol induced a G1 phase arrest in human gastric adenocarcinoma and murine melanoma cells (34) and in mouse mammary tumor cells (35). Recent reports have also established that γ - and δ -tocotrienols, and to a lesser extent α -tocotrienol, were able to induce apoptosis in breast cancer, hepatocellular, and melanoma cells (5). Similarly, tocotrienols proved their effective anticancer activity against prostate cancer where in their study, Luk and colleagues demonstrated γ -tocotrienol to prevent chemoresistance by eliminating prostate cancer stem cells in androgen-independent pathway (36). Furthermore, recent *in vitro* studies in the highly malignant+SA mammary tumor cell line demonstrated an inhibitory effect of γ -tocotrienol on Met expression and activation where γ -tocotrienol significantly inhibited the HGF-dependent+SA cell replication (37), an effect that was further enhanced by its combined treatment with SU11274, a Met inhibitor, in various mammary cancer cell lines (38). Consistent with the anticancer activity observed *in vitro*, when administered intraperitoneally, γ -tocotrienol showed significant life-prolonging effect in mice implanted with different types of cancers, including inoculated Ehrlich carcinoma, Sarcoma 180, IMC carcinoma, and Meth A fibrosarcoma, at doses of 20, 5, 5, and 40 mg kg⁻¹ day⁻¹, respectively, an effect that was higher than that observed with α -tocotrienol (39). However, both isoforms were inactive in P388 leukemia in the dose range 5–40 mg kg⁻¹ day⁻¹. A previous *in vitro* study has investigated the relative potency of various tocotrienol isoforms against breast cancer cells where α -, γ -, and δ -tocotrienol isoforms and TRF were able to inhibit the growth of the 4T1 murine mammary cancer cells and exhibited an antiproliferative effects in the following order: δ -tocotrienol> γ -tocotrienol>TRF> α -tocotrienol (40).

Tocotrienols have also shown to possess an inhibitory effect on cancer cells invasion and metastasis. For example, γ -tocotrienol has shown to have beneficial effect in preventing

the spread of gastric cancer cells, demonstrating the inhibitory effects on cell migration and cell Matrigel invasion in the concentration range 15–60 μ M. γ -Tocotrienol significantly reduced the Matrigel invasion capability of the gastric adenocarcinoma SGC-7901 cells through down-regulation of the mRNA expressions of matrix metalloproteinase-2 and metalloproteinase-9, and upregulation of tissue inhibitor of metalloproteinase-1 and metalloproteinase-2 when compared with the control (41). Furthermore, a recent study has examined the possibility of using TRF as an adjuvant to enhance the anticancer effects of dendritic cell-based cancer vaccine in a syngeneic mouse model of breast cancer (42). The combined therapy of using dendritic cells pulsed with tumor lysate from 4T1 cells injections and TRF supplementation was able to inhibit tumor growth and metastasis (42).

Human Clinical Studies

Clinically, it has been reported that women who developed breast cancer had significantly lower plasma levels of α -tocopherol compared with controls (43). In addition, findings from a large randomized trial conducted to determine if micronutrients could impact the incidence of esophageal, stomach, and overall cancers demonstrated that when grouped with β -carotene and selenium, α -tocopherol had a significant benefit on total mortality when compared with placebo group (43). Also and as part of a randomized, double-blind, placebo-controlled trial called Alpha-Tocopherol, Beta Carotene (ATBC), which investigated the effect of antioxidant supplements on the incidence of lung cancer, male smokers received 50 mg of α -tocopherol daily for 5 to 8 years were found to have significantly reduced incidence of prostate cancer and mortality (44). Furthermore, a meta-analysis of 12 randomized control trials in analyzed vitamin E data, given as a supplement, concluded that vitamin E can be used as a preventive therapy in men who are at high risk of prostate cancer (45). In addition to its anticancer activity against prostate cancer, findings from the New Hampshire Study suggested that higher total intake of dietary vitamin E was able to reduce the risk of bladder cancer (46). With regard to protective effect of tocotrienols against cancer, previous reports have shown the consumption of palm oil containing diet-increased tocotrienol levels in breast adipose tissues of patients compared with tocopherols (47). This higher level of tocotrienols could be related to their ability to better transfer across and incorporation into the membranes than tocopherols (48). Findings from these studies demonstrated a significant difference in the total tocotrienol levels between malignant and benign adipose tissue samples, being higher in the later, suggesting tocotrienols' protection effect against breast cancer (47,48).

While several clinical studies demonstrated the anticancer and preventive activities of vitamin E supplementation, few clinical studies reported the lack of such association. For example, findings from the Iowa Women's Health Study (IWHs) were not in agreement with the above results, and the investigators concluded that vitamin E intake did not decrease the incidence of breast cancer (49). In another study, measured levels of the lipid-soluble α -tocopherol in tissue samples of female patients with breast and gynecologic malignant cancers, showed a heterogeneity in origin and type

of cancer and α -tocopherol levels with a significant positive correlation with cervical and endometrial cancers (50). Similarly, findings from the randomized clinical trial Selenium and Vitamin E Cancer Prevention Trial (SELECT) showed that α -tocopherol supplementation increased the incidence of prostate cancer (51).

VITAMIN E AS A CHEMOSENSITIZER IN CANCER THERAPY

Multiple drug resistance (MDR) is a major clinical problem affecting the successful and effective chemotherapeutic treatment of cancer. Cancer cells display MDR through multiple mechanisms. One of the most common mechanisms is the overexpression of various members of the ABC family of transport proteins (52). P-glycoprotein (P-gp) is the most-studied ABC transporter that efflux out several chemotherapeutic agents, including anticancer drugs, from cells and thereby reducing their intracellular accumulation in drug-resistant cells (52).

P-gp inhibition, thus, is expected to increase the efficacy of chemotherapeutic agents in MDR cells. D- α -tocopheryl

polyethylene glycol succinate (TPGS), a derivative of α -tocopherol, is one of the most potent and commercially available surfactants, which also acts as a P-gp inhibitor (53). TPGS modulates the efflux pump activity of P-gp via several mechanisms, including (i) competitive inhibition of substrate binding; (ii) alteration of membrane fluidity, and/or (iii) inhibition of the efflux pump ATPase activity (54). TPGS has shown to modulate the sensitivity of several antitumor agents *in vitro* and *in vivo*. It has been reported that the activity of P-gp was decreased and the nuclear accumulation and cytotoxicity of doxorubicin were significantly increased in drug-resistant breast cancer cells after incubation with nanoparticles containing TPGS (54). The cytotoxicity and antitumor efficacy of paclitaxel prepared as nanocrystals containing TPGS were significantly higher when compared with Taxol® and achieved better therapeutic effect in paclitaxel-resistant cancer cells in both *in vitro* and *in vivo* models (55). In addition, when tested in MDR cancer cells, TPGS-emulsified nanoparticles significantly enhanced the intracellular accumulation and cytotoxic effect of SN-38, active metabolite of irinotecan, as a result of P-gp inhibition

Fig. 2. The role of transport proteins in the intestinal absorption and hepatic disposition of vitamin E isoforms (E)

(56). Furthermore and as part of targeted delivery system for docetaxel, TPGS increased the *in vitro* efficacy of docetaxel by approximately 200- and 220-fold higher than Taxotere® in MDA-MB468 and MDA-MB231 breast cancer cell lines, respectively (57). These findings were further supported by a recent *in vivo* study using xenograft BALB/c nude mice tumor model where the cytotoxicity and cellular uptake of docetaxel were significantly enhanced when encapsulated in nanoparticles that contain TPGS (58).

In addition to its anticancer activity, described above, the synthetic form of α -tocopherol, TS, may also act as an MDR inhibitor. Treatment of two human MDR cell lines (H460/taxR and KB-8-5) with TS exhibited a significant reversal of the MDR effect compared with paclitaxel alone; however, the inhibition effect was less than that observed with TPGS (59). In agreement with the *in vitro* findings, the *in vivo* studies using MDR cell xenograft model showed the ability of TS to overcome MDR when simultaneously administered with paclitaxel (59). The mechanism by which TS enhanced paclitaxel efficacy was explained by its inhibitory effect on the ATPase activity resulting in increased intracellular accumulation of paclitaxel, without altering the levels of P-gp expression (59). These findings suggest that derivatives of vitamin E could be used in combination with anticancer drugs and/or as part of the delivery system for MDR sensitization.

SYSTEMIC AVAILABILITY OF VITAMIN E ISOFORMS

α -Tocopherol is considered the predominant form of vitamin E in plasma and tissues of humans and animals. The plasma concentration of tocotrienols and other tocopherols is much lower than that of α -tocopherol. Previous studies showed that γ -tocopherol concentrations are much lower than those of α -tocopherol in rats (1.3–1.7 vs. 7.2–13.0 $\mu\text{mol/l}$) (60,61). In humans, the plasma concentration of γ -tocopherol was four to ten times lower than α -tocopherol (62). At baseline and following 6 h of fasting, the plasma concentrations of α -, sum of β - and γ -, δ -tocopherols, and α -, γ -, and δ -tocotrienols in humans were 1070, 120, 4, 0, 0, and 11 $\mu\text{g/dl}$, respectively (63). These concentrations were significantly increased after treatment with supplementary capsules containing 80 mg of tocotrienols (26.5 mg of α -, 36.6 mg of γ -, and 16.9 mg of δ -tocotrienol) together with 64 mg of α -tocopherol for 10 days to reach 1940, 54, 18, 16, 13, and 12 $\mu\text{g/dl}$ in 2-h postprandial plasma (63).

The ability of vitamin E isoforms to elicit the desired pharmacological response and to be used effectively in cancer therapy depends on their availability at the target site, which in turn is influenced by the plasma concentrations. However, reported poor oral bioavailability of vitamin E isoforms limits their utilization as therapeutic agents in cancer therapy. The limited bioavailability of vitamin E and isoforms could be attributed to the following reasons: (1) vitamin E members are lipophilic in nature that are practically insoluble in water and display high solubility in organic solvents (about 10 $\mu\text{g/ml}$) (64,65), and (2) available studies, including ours, have reported vitamin E isoforms as substrates for intestinal transport proteins, which become saturated in the presence of high concentrations, thus limiting their oral bioavailability and prompting nonlinear absorption kinetics (66,67).

In humans, although the absolute bioavailability was not determined, γ -tocotrienol-relative bioavailability increased 3.5-fold when administered with food (68), whereas in rats, δ - and γ -tocotrienol oral bioavailability was determined to be as low as 8.5% and 9%, respectively (65,66). On the other hand, the oral bioavailability of α -tocopherol was significantly higher than both tocotrienols with 36% when tested in rats (64).

ROLE OF TRANSPORT PROTEINS IN THE DISPOSITION OF VITAMIN E ISOFORMS

The disposition of vitamin E isoforms is controlled by different transport proteins, especially those expressed in the enterocytes that determine their systemic bioavailability (Fig. 2). This part will focus on role of transport proteins in the intestinal absorption and hepatic disposition of tocopherols and tocotrienols.

Intestinal Absorption

Tocopherols and tocotrienols are lipophilic molecules, therefore requiring utilization of several processes necessary for fat absorption. Vitamin E isoforms should be in the solubilized form for intestinal absorption. The secretion of pancreatic esterases and bile acids in the intestinal lumen are vital for the micellization of vitamin E isoforms and lipid hydrolysis products. The formed micelles are absorbed by intestinal enterocytes. Then, in the enterocytes, the isoforms are incorporated into chylomicrons which consequently are secreted into the lymphatic system and finally reaching the plasma (69).

Although the intestinal uptake of vitamin E isoforms was initially described as a passive process, several studies have recently established the role of active transport processes in their intestinal absorption. Numerous studies have been conducted to identify transporters responsible for vitamin E intestinal uptake. Reboul et al. reported that only a fraction of α -tocopherol was absorbed by passive diffusion because of the 30–50% absorption observed at 4°C, while the remaining was absorbed by a receptor mediated process (70). To identify the involved receptor, the authors performed *in vitro* inhibition studies and found that the protein scavenger receptor class B type 1 (SR-B1) expressed in Caco2 cells as well as in the enterocytes is responsible, in part, in determining the intracellular levels of tocopherols. This role of SR-B1 in the uptake of tocopherols was further confirmed by inhibition studies where the cellular uptake of α - and γ -tocopherols was reduced by SR-B1 antibody and BLT1, a specific inhibitor of SR-B1 (70).

Based on the fact that the transport protein Niemann-Pick C1-like 1 (NPC1L1) is critical for cholesterol intestinal absorption, and the assumption that fat-soluble molecules could share similar absorption mechanism (71), Narushima and his colleagues studied the role of NPC1L1 in the transport of α -tocopherol (72). The authors *in vitro* and *in vivo* findings suggested and confirmed NPC1L1 mediates the uptake of α -tocopherol that was reduced by ezetimibe, a specific inhibitor of NPC1L1 (72). A recent study focused on investigating the respective contribution of NPC1L1 and SR-B1 to the intestinal absorption of tocopherols (73). The *in vitro* findings of this study with Caco2-TC-7 cells confirmed γ -tocopherol uptake to be mediated by both SR-B1 and

NPC1L1, which was reduced by 30% and 70% when co-treated with BLT1 and ezetimibe, SR-B1, and NPC1L1 inhibitors, respectively (73). Consistent with the *in vitro* data, results from the *in vivo* studies in mice overexpressing intestinal SR-B1 and in wild-type mice demonstrated role for both proteins in the intestinal absorption of γ -tocopherol. In addition, findings from these experiments showed differential distribution expression of both proteins where in wild-type mice the absorption of γ -tocopherol was observed in the distal jejunum that was specifically inhibited by ezetimibe indicating NPC1L1 expression, while in the SR-B1-overexpressing mice, γ -tocopherol absorption increased in the proximal part of the intestine (73).

Studies from our laboratory showed that tocotrienols as substrates for NPC1L1 transport protein. Using an *in situ* rat intestinal perfusion model, we showed that the intestinal uptake of γ -tocotrienol is concentration-dependent and a saturable process. Results from the *in situ* and additional *in vitro* inhibition studies revealed the significant contribution of NPC1L1 to the uptake and transport of γ -tocotrienol across the cell membrane (66,67). The *in vitro* interaction kinetics of γ -tocotrienol with NPC1L1 was further examined in NPC1L1-transfected cells and was compared with those of α -tocopherol. Our results confirmed that both isoforms are substrates of NPC1L1 and that α -tocopherol is transported at significantly higher rates, however with lower affinity, compared with γ -tocotrienol, which could be due to the higher passive permeability of α -tocopherol (64). In addition, *in vitro* and *in vivo* studies performed by us demonstrated that like the γ -isoform, δ -tocotrienol transport is mediated by NPC1L1 and that the intestinal absorption of both tocotrienols was capacity limited and saturable, thus limiting their bioavailability (66,67).

It has long been assumed that following absorption, vitamin E isoforms are incorporated into chylomicrons as non-esterified molecules and secreted into the lymph via apolipoprotein-dependent pathway (74). However, several *in vitro* and *in vivo* studies have demonstrated a role for the basolateral ATP-binding cassette A1 (ABCA1) transporter in the secretion of vitamin E to the blood of portal vein. *In vitro* studies by Reboul et al. showed that tocopherols efflux was induced by apolipoprotein A-I (apoA-I) added to the basolateral side of Caco2 monolayer (75). This efflux was impaired by glyburide, a commonly used inhibitor of ABCA1 transporter (75). In addition, the same authors reported that the postprandial response of plasma γ -tocopherol was 4-fold lower in ABCA1^{-/-} mice than in wild-type mice, and the fasting plasma levels of α -tocopherol were lower in mice bearing the genetic deletion (75).

Collectively, findings from *in vitro*, *in situ*, and *in vivo* studies support the roles for NPC1L1, SR-B1, and ABCA1 in the intestinal absorption and plasma levels of vitamin E isoforms. However, further studies are required to elucidate the relative contribution of each of these proteins to the absorption of various isoforms of vitamin E.

Hepatic Disposition

When reaching the blood circulation, chylomicrons containing vitamin E must undergo degradation for further processing. Circulating chylomicrons undergo triglyceride lipolysis by lipoprotein lipase (LPL) secreted by the pancreas to form free fatty acids, vitamin E-containing lipoproteins,

chylomicron remnants, or LDL and HDL containing vitamin E that can be taken up by tissues and/or by the liver. Vitamin E is readily transferred between HDL and other lipoproteins with the help of phospholipid transfer protein (PLTP). The chylomicron remnants are taken up by the liver and incorporated into VLDL by α -tocopherol-transfer protein (α -TTP) and subsequently transported to the various peripheral tissues (69). Previous studies have determined that α -tocopherol is preferentially secreted by α -TTP into the plasma for distribution to the peripheral tissues (76). The other isoforms of vitamin E have a much lower affinity for α -TTP which makes these isoforms' levels in the plasma much lower than that of α -tocopherol (69). The distribution of vitamin E isoforms varies between different tissues. Previous studies conducted in mice and hamster reported that tocopherols and tocotrienols were detected in all tissues including heart, liver, and kidney (63,77). However, in the brain, only tocopherols could be detected but not tocotrienols (70), which could be related to sensitivity of the analysis method. In addition, tocotrienols were found to penetrate and distribute rapidly through skin (78).

In human plasma, approximately half of the α -tocopherol is incorporated into HDL with the other half into LDL and VLDL (69). Several studies have been conducted to study the mechanism involved in mobilizing α -tocopherol from cells into the HDL pathway. Shichiri et al. investigated the re-efflux of α -tocopherol from hepatocytes, the cells that have the most important role in regulating plasma- α -tocopherol concentrations. In their *in vitro* studies using hepatoma cell line stably expressing α -TTP, the authors observed that the addition of apoA-I, a direct acceptor of the ABCA1-secreted lipids, increased α -tocopherol secretion in a dose-dependent manner (79). This effect was abolished by the addition of probucol, an ABCA1 inhibitor, where the cellular secretion of α -tocopherol was significantly reduced (79). This finding was supported by *in vivo* studies where the addition of 0.1% probucol to mice diet for 4 weeks significantly decreased the plasma levels of murine α -tocopherol by >80% (79). To further confirm the role of ABCA1 in α -tocopherol efflux, ABCA1-RNAi experiments were performed, and consistent with the above inhibition studies, ABCA1 knock-down reduced the hepatic secretion of α -tocopherol (79). Collectively, these findings confirm role for ABCA1 in the intestinal and hepatic secretion of α -tocopherol and distribution into plasma and peripheral tissues. However, further investigations are required to elucidate the role of ABCA1 in tocotrienols disposition.

Although human dietary intake of other vitamin E isoforms is sometimes higher than that of α -tocopherol, human plasma and tissue levels of α -tocopherol are significantly higher than the other isoforms (3). The accumulation of α -tocopherol in tissues and the higher plasma levels is due to the selective binding to hepatic α -TTP and the hepatic regulation of vitamin E metabolism and excretion (14). All vitamin E isoforms are metabolized along the same pathway via phase I and phase II metabolizing enzymes (9). Cytochrome P-450 (CYP) enzymes are involved in the oxidation of the side chain, with CYP3A4 and CYP4F2 are the most likely candidates (9). Tocopherols and tocotrienols are metabolized to α -, β -, γ -, and δ -carboxyethyl hydroxychromans (CEHC) by side chain degradation,

initially ω -hydroxylation by CYP4F2 or 3A4, followed by five β -oxidation cycles (14). The second and the fourth cycles of β -oxidation of tocotrienols side chain require 2,4-dienoyl-CoA reductase and 3,2-enoyl-CoA isomerase, respectively (80). Then, CEHCs undergo enzymatic conjugation catalyzed by either sulfotransferases (SULTs) to produce sulfate esters or by glucuronosyl transferases (UGTs) which produce glucuronide conjugated products before their biliary and/or urinary excretion (14). In addition to the metabolism and formation of CEHCs, vitamin E itself is excreted unchanged into bile. The mechanism by which α -tocopherol is secreted into the bile was studied by Mustacich and colleagues. The authors demonstrated that the biliary excretion of α -tocopherol is mediated by the efflux transporter P-gp (81). Role for P-gp in the efflux of tocopherols and tocotrienols was further examined by molecular docking (82). The docked conformation of tocotrienols with P-gp revealed α - and δ -tocotrienols to have highest binding affinity compared with the β - and γ -isoforms, and that tocotrienols better interact with P-gp than tocopherols (82). While these data support vitamin E as possible P-gp substrates, further studies using various experimental models are required to clarify P-gp role in vitamin E hepatic disposition.

To our knowledge, studies elucidating the role of NPC1L1 localized at the canalicular membrane of hepatocytes in the hepatic disposition of vitamin E isoforms are lacking. Compared with intestinal NPC1L1, the function of hepatic NPC1L1 in humans remains largely unknown (83); however, it is expected for this protein to regulate tocopherols and tocotrienols hepatic levels possibly via their reuptake from the bile back to the hepatocytes. Investigations that focus on clarifying the function of hepatic NPC1L1 and its possible interplay with P-gp in regulating vitamin E isoforms levels are vital to identify strategies for improved disposition and enhanced bioavailability.

STRATEGIES TO IMPROVE VITAMIN E ORAL BIOAVAILABILITY

Major factors that determine the amount of an absorbed drug following its oral administration are dissolution rate in the intestinal lumen and/or the permeability across the intestinal cells (84). As described earlier, vitamin E isoforms suffer from both, poor solubility and permeability. Thus, to overcome such limitations and enhance the oral bioavailability, an approach that enhances the solubility and permeability is essential.

One of the promising strategies to enhance the absorption of poor water-soluble drugs is the utilization of drug-delivery systems. Several studies have been conducted to investigate the possibility of using drug-delivery systems to enhance the solubility and permeability of vitamin E isoforms. Packaging γ -tocotrienol in solid lipid nanoparticles (SLN) as a model formulation was tested for its effect on γ -tocotrienol permeability and bioavailability (85). *In situ* studies demonstrated SLN increased the permeability of γ -tocotrienol by 10-fold when compared with mixed micelles, representing the end product of intestinal digestion that is most readily available for absorption and was used as control. Subsequent *in vivo* studies showed that γ -tocotrienol-relative oral bioavailability from SLN was 3-fold higher than mixed

micelles (85). Further studies investigated the incorporation γ - and δ -tocotrienol in self-emulsifying drug-delivery systems (SEDDS) on their bioavailability. Findings of these studies showed a significant increase in the solubility, permeability, and oral bioavailability of γ - and δ -tocotrienols both *in vitro* and *in vivo* (67,86). To explain the enhanced intestinal permeability and bioavailability of γ - and δ -tocotrienol delivered as either SLN or SEDDS compared with their delivery as mixed micelles, *in vitro* mechanistic studies were performed (67,85,86). Results of these studies revealed that packaging γ - and δ -tocotrienol in SLN or SEDDS delivery systems were able to enhance both isoforms' intestinal permeability and bioavailability as a result of the enhanced passive permeability and reduced contribution of NPC1L1 to the transport of both isoforms (67,85,86). One of the possible explanations for the increased passive permeability by SLN and SEDDS could be related to the excipients incorporated in these delivery systems and their ability to modulate the fluidity of cell membrane (67,85,86). Similar findings were observed with Yap et al.'s study (73), who reported the delivery of tocotrienols in SEDDS formulation caused faster onset of absorption with marked increase in the bioavailability of tocotrienols by 2- to 3-fold in humans (87). The authors explained the enhanced oral bioavailability by the sufficient droplet size, in submicron range, of the emulsion product formed as well as the enhanced rate and extent of lipolysis (87). Furthermore, Miyoshi et al. investigated the effect of TRF inclusion complex with cyclodextrin (CD) on the oral bioavailability of γ -tocotrienol in mice (88). The oral administration of TRF/CD complex significantly increased γ -tocotrienol plasma levels with 1.4-fold increase in its relative oral bioavailability compared with TRF-administered group. In addition, TRF/CD complex allowed rapid absorption of γ -tocotrienol at 3 h compared with 6 h following TRF administration (88).

Recently, an interesting strategy was reported to co-deliver paclitaxel and TS in chitosan derivative polymeric micelles to SK-OV3 ovarian carcinoma cell line (89). The incorporation of TS into paclitaxel-loaded micelles produced smaller particles with significant higher stability during storage without affecting the entrapment efficiency of paclitaxel into the copolymer. In addition, cell cytotoxicity studies demonstrated the cytotoxic effect of TS that acted synergistically with paclitaxel, which represented a new combinatory strategy for efficient delivery and efficacy (89).

CONCLUSIONS

In this review, we have described the anticancer activity of vitamin E isoforms and factors affect their body disposition and oral bioavailability. While several factors contribute to their low bioavailability, poor absorption of vitamin E isoforms across the intestinal membrane has been reported to play important role in determining this low bioavailability. The oral absorption of tocopherols and tocotrienols into the blood circulation is mediated, in large part, by transport proteins, mainly SR-B1 and NPC1L1, which display saturation when exposed to high concentrations. To improve their systemic bioavailability, delivery systems that could successfully enhance the passive permeability of tocopherols and tocotrienols, and minimize the carrier-mediated transport

processes were developed. In addition to SR-B1 and NPC1L1, it is expected that intestinal and hepatic function of ABCA1 to play important role in regulating α -tocopherol, and possibly other isoforms, plasma levels, and thus bioavailability.

In addition, examples of the potential benefit of vitamin E derivative-based delivery systems and/or in combination with chemotherapeutic agents in the treatment of cancer have been described both *in vitro* and when available *in vivo*. These examples clearly illustrate the promise of vitamin E isoforms and derivatives as novel anticancer treatments in the future. However, several challenges remain yet to be resolved to speed up their utilization as anticancer therapeutics. Example of these challenges is the low systemic bioavailability of vitamin E isoforms, which is considered a major issue to maintain their blood concentrations within therapeutic levels for cancer prevention and/or treatment. Thus, efficient drug-delivery systems are essential to deliver the right concentrations of these isoforms. In addition, there is a need for well-controlled clinical studies to investigate the effect of vitamin E isoforms in various cancer types, the clinical relevant doses and therapeutic concentrations, the concentrations of vitamin E isoforms in tumor tissues, and the mechanisms involved in the uptake of vitamin E isoforms into these tumor tissues.

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