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REVIEWS: CURRENT TOPICS

Antiangiogenic and anticancer potential of unsaturated vitamin E (tocotrienol)

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Abstract

Several lines of evidence support the beneficial effect of tocotrienol (T3; an unsaturated vitamin E) on inhibition of tumor development. Many factors, including decrease in oxidative stress and modulation of cell signaling pathways in tumor and endothelial cells, have been implicated in such anticancer action of T3, while the in vivo potency and exact intracellular mechanisms for the anticancer properties of T3 remain not fully understood. We have hypothesized that the inhibitory effect of T3 on cancer may be attributable to the antiangiogenic activity of T3, and we found that T3 acts as a potent regulator of growth-factor-dependent signaling in endothelial cells and as an antiangiogenic agent minimizing tumor growth. In this work, we review the history and biological action (i.e., anticancer) of vitamin E and describe current research on the antiangiogenic effects of T3 and its mechanisms.
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Keywords: Antiangiogenesis; Endothelial cells; Tocotrienol; Tumor; Vitamin E

1. Introduction

1.1. Historical aspect, structure and distribution of vitamin E

In 1922, vitamin E was discovered in green leafy vegetables as a micronutrient essential for reproduction [1]. Because vitamin E supported fertility, it was scientifically named tocopherol (Toc) [2]. The word "tocopherol" comes from the Greek words *tokos* (meaning childbirth), *phero* (meaning to bring forth) and *ol* (indicating the alcoholic properties of this molecule). Eight substances have been found in nature as vitamin E: four Tocs [RRR-α-tocopherol (α-Toc), RRR-b-tocopherol (b-Toc), RRR-γ-tocopherol (γ-Toc) and RRR-d-tocopherol (d-Toc)] and four tocotrienols (T3) [α-tocotrienol (α-T3), β-tocotrienol (β-T3), γ-tocotrienol (γ-T3) and δ-tocotrienol (δ-T3)] (Fig. 1). Structurally, vitamin E consists of a chroman head with two rings (one phenolic acid ring and one heterocyclic ring) linked to an isoprenoid-derived hydrophobic tail. The aliphatic tail of Toc is fully saturated, while the side chain of T3 contains three *trans* double bonds. Four different forms of each Toc and T3 differ in the numbers and positions of methyl groups attached to the chroman head. α, β, γ and δ forms of Toc and T3 are often referred to collectively as tocochromanols. The tocochromanols are synthesized in plastids of plants from precursors derived from the shikimate and methylerythritol phosphate pathways [3]. Toc presents in various foods such as vegetable oils and nuts, while T3, a minor plant constituent, is abundant especially in rice bran, palm oil and annatto seeds [4,5].

1.2. Physiological activity and bioavailability of Toc and T3

Toc and T3 are classified based on their ability (vitamin E activity) to prevent the resorption of rat fetuses. α-Toc displays the highest efficacy among the eight tocochromanols, whereas α-T3 has about one third of the activity of α-Toc. Regardless, all forms of tocochromanols are able to induce antioxidative effects and to act as protective agents against lipid peroxidation in biological membranes. In some model membrane studies,

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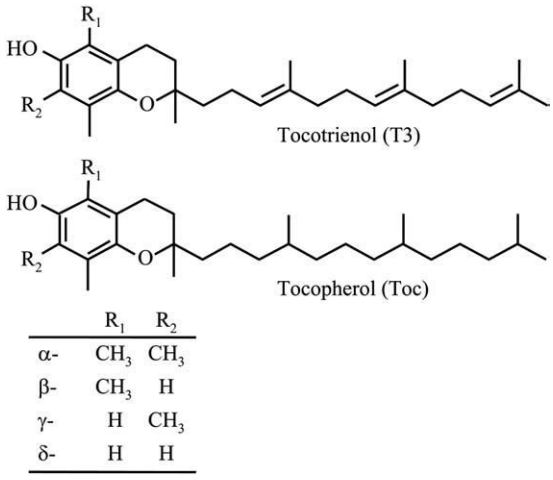

	R_1	R_2
α-	CH_3	CH_3
β-	CH_3	H
γ-	H	CH_3
δ-	H	H

Fig. 1. Chemical structures of Toc and T3.

T3 has been reported to be a more potent antioxidant than Toc [6]. Moreover, T3 has recently gained increasing scientific interest due to its several health-promoting properties that differ somewhat from those of Toc [7]. For example, micromolar amounts of T3 suppress the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the hepatic enzyme responsible for cholesterol synthesis [8]. T3 protects neuronal cells against oxidative damage [9]. Furthermore, T3 has been documented to have potent abilities to induce cell cycle arrest [10], to activate p53 and caspase [11,12], to suppress adhesion molecules [13], to inhibit nuclear factor-κB [14] and to down-regulate c-Myc and telomerase [15]. These unique effects of T3 could be partly explained by its absorption and metabolic fate in vivo. Although absorption mechanisms are basically the same for all tocochromanols, T3 has been reported to be absorbed preferentially into human liver carcinoma (HepG2) cells [16] and to be degraded to a greater extent than Toc in rats [17].

On the other hand, the bioavailability of orally taken T3 is relatively inferior to that of α-Toc [18]. Hepatic α-Toc transfer protein (α-TTP) is a critical regulator of vitamin E in mammals, and it selects α-Toc from tocochromanols in the liver, facilitating α-Toc secretion into nascent very-low-density lipoproteins [19]. Although affinity between α-TTP and T3 is low, it has been shown that T3 orally administered to humans is absorbed from the intestine to the blood, and plasma T3 concentration reaches about 1 μM [20]. Of note, such circulating level of T3 is almost an order of magnitude higher than that required to protect neurons against a range of neurotoxic insults in cell culture studies [9].

1.3. T3 and cancer

Besides the above-mentioned health-promoting properties, several lines of evidence support the beneficial effect of T3 on inhibition of tumor development (for details, refer to Cancer Chemoprevention by T3). One of the studies investigating the role of T3 in neoplastic disorders reported that α-T3 and γ-T3 effectively suppressed the development of sarcoma 180, Ehrlich carcinoma and invasive mammary carcinoma [21]. In addition, in a rat mammary tumor model, only T3 enhanced tumor latency (an indicator of efficacy), while Toc did not [22]. Subsequent cell culture studies reported that T3 suppresses the proliferation of various tumor cells, including colon, breast and prostate cancer cells [10,15,23]. Some recent animal studies showed that T3 inhibits liver and lung carcinogeneses, and suppresses the growth of breast tumors and melanoma [24–27]. Many factors, such as decrease in oxidative stress and modulation of cell proliferation signaling pathways (including apoptosis induction), have been implicated in the anticancer action of T3 in tumor cells.

1.4. T3 and angiogenesis

Angiogenesis is the formation of new blood vessels from preexisting endothelium and is closely involved in cancer progression [28]. In angiogenesis, endothelial cells secrete proteases, migrate through the extracellular matrix, proliferate and differentiate [29]. The final step is the formation of blood vessels newly fused with vascular smooth muscle cells, leading to blood flow into the tumors. Angiogenesis starts with tumor cells releasing specific angiogenic molecules [e.g., vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF)] that further activate angiogenic gene expression in endothelial cells and enhance vascular permeability [30]. An understanding of the fundamental mechanisms of angiogenesis has allowed the discovery of inhibitors targeting VEGF for use in cancer therapy, as well as in the prevention of angiogenic disorders such as diabetic retinopathy [31]. Several antiangiogenic drugs (e.g., ZD6474 and vatalanib/PTK787) are currently being studied in clinical trials involving patients with a wide variety of cancers, and some of these agents have considerable promise for future treatments [32,33]. Since some antiangiogenic agents are available in foods [34–39], even if these agents possess moderate antiangiogenic effect, daily consumption of these compounds may help prevent angiogenic disorders.

On the basis of this background, it is of high interest to know whether T3 suppresses cancers through its suppressive effect on tumor angiogenesis. We have hypothesized that the inhibitory effect of T3 on cancer may be attributable to the antiangiogenic activity of T3, and we have carried out a series of investigations [40–44]. We found that T3 acts as a potent regulator of growth-factor-dependent signaling in endothelial cells and as an antiangiogenic agent minimizing tumor growth. In this review, we introduce knowledge about cancer prevention by T3 and describe current research on the antiangiogenic effect of T3 and its underlying mechanisms.

2. Cancer chemoprevention by T3

2.1. T3 inhibits cancer cell proliferation

Cancer is one of the leading causes of death, and nutritional factors play an important role in the prevention

or occurrence of cancer. Many previous studies have suggested vitamin E as one of candidates for the adjuvant treatment of tumorigenesis [45]. Even though early studies focused on Toc, recent evidence suggests that T3 displays better antitumor property than Toc by inhibiting the growth and proliferation of many cancerous cells such as breast, colon, liver and prostate cancer cells [15,23,46]. For instance (e.g., in vivo study), dietary supplementation of T3 showed inhibition of the growth of estrogen-receptor-positive human breast cancer cells in athymic nude mice [47]. In a study of C57BL mice with implantation of melanomas, a T3-rich diet delayed tumor growth and prolonged the survival rate [24]. Intraperitoneal administration of T3 (α-T3 and γ-T3), but not Toc (α-Toc), showed a life-prolonging effect in mice transplanted with tumors [21]. Long-term supplementation of a T3-rich diet reduced the activity of alkaline phosphatase (a marker of neoplastic transformation) in rats [27,48]. These in vivo studies supported the anticancer activity of T3. A number of studies in the literature suggested various possible mechanisms for the anticancer action of T3, including decrease in oxidative stress, activation of p53, modulation of Bax/Bcl-2 ratio and induction of apoptosis [11]. More recent studies have shown that T3 (γ-T3) inhibits cancer cell proliferation by decreasing Akt phosphorylation and by activating NF-κB [49], indicating its ability to regulate cell growth, cell cycle and apoptosis. In addition, T3 has been reported to inhibit several enzymes related to cancer cell proliferation, including DNA polymerase and telomerase [15,50]. Among these possible mechanisms, apoptosis induction is considered to play a major role in the antiproliferative effect of T3.

2.2. T3-induced apoptosis

Apoptosis induction is arguably the most potent defense against cancer progression. Indeed, most of the currently used chemotherapeutic drugs inhibit cancer cell proliferation by inducing apoptosis. Many researchers have investigated the effects of T3 on normal and cancer cells and have revealed that T3 induces apoptosis preferentially in cancer cells [10,51]. For instance, T3 showed potent apoptotic activity in preneoplastic, neoplastic and highly malignant mouse mammary epithelial cells [52] and, at the same dose, T3 had almost no adverse effects on the growth or function of normal counterpart cells.

Although the exact cell death signaling events by T3 have not yet been fully elucidated, many studies have been conducted to elucidate its apoptotic mechanism. Sakai et al. [53] reported that caspase-3, caspase-8 and caspase-9 were involved in T3 (γ-T3)-induced apoptosis of Hep3B cells, and that Bax and Bid participated in the regulation of apoptosis induction. T3 also enhances caspase-8 activity in highly malignant +SA mouse mammary epithelial cells and rat hepatoma dRLh-84 cells [12,54]. T3-induced caspase-9 expression was also reported in human colon carcinoma cells via activation of p53 and increase in Bax/Bcl-2 ratio [11]. However, another study reported that T3 induced the expression of caspase-8, but not caspase-9, in murine mammary cancer cells [54–56]. T3 (δ-T3)-induced apoptosis has also been reported to be involved with the activation of transforming growth factor-β-, Fas- and JNK-signaling pathways in human breast cancer cells [57]. A more recent study by Ahn et al. [14] showed that γ-T3 inhibited the NF-κB activation pathway through inhibition of RIP and TAK1, leading to suppression of antiapoptotic gene products and potentiation of apoptosis. Although further studies must be required to completely clarify the mechanism for T3-induced apoptosis, the evidence discussed above, at any rate, confirms that T3 can actually induce apoptosis in cancer cells, which would be attributable, in part, to the inhibitory effect of T3 on tumor growth.

3. Antiangiogenic properties of T3

3.1. Modulation of angiogenesis for cancer prevention

Recently, there has been increasing evidence that tumor growth is dependent principally on angiogenesis (formation of new blood vessels) [58]. The newly formed blood vessels can promote cancer growth by supplying nutrients and oxygen and by removing waste products. Metastasis also depends on angiogenesis, as tumor cells are shed from a primary tumor and grow at their target organs [59]. Considering these perspectives, antiangiogenic therapy is a promising approach in the treatment of cancer and other proangiogenic diseases. In addition to the direct inhibitory effect of T3 on tumor cell growth (refer to Cancer Chemoprevention by T3), our recent works demonstrated the indirect effect of T3 (i.e., inhibition of angiogenesis for cancer prevention) [40–44].

3.2. In vitro study of angiogenesis inhibition by T3

Since angiogenic processes are involved in endothelial cell proliferation, migration and tube formation, modulation of these processes serves as a good strategy for preventing angiogenic disorders. Our cell culture studies [40–44], which aimed to screen for food-derived antiangiogenic compounds by assessing the proliferation, migration and tube formation of endothelial cells, showed T3 to be a potential angiogenesis inhibitor. All T3 isomers at a low micromolar range inhibited the proliferation, migration and tube formation of bovine aortic endothelial cells (BAEC) and human umbilical vein endothelial cells (HUVEC) (Fig. 2), with the following order of inhibitory potency: δ-T3>β-T3>γ-T3>α-T3 [40,43]. In contrast, Toc did not exhibit any effect on the proliferation, migration and tube formation of BAEC and HUVEC. These findings suggested that T3, but not Toc, has considerable potential as an angiogenesis inhibitor. Structurally, T3 and Toc can be distinguished by considering their side chains (Fig. 1), and it has been reported that the unsaturated side chain of T3 allows it to pass through cell membranes more efficiently at a rate faster than that of the saturated phytyl side

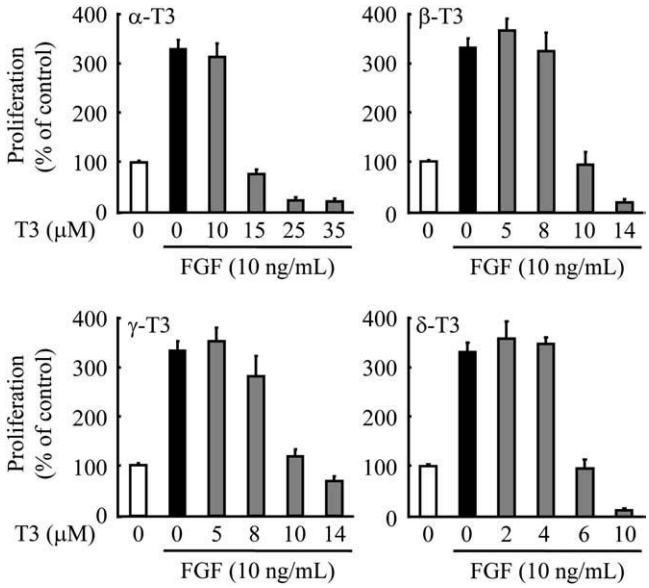

Fig. 2. Effects of T3 on FGF-induced HUVEC proliferation. HUVEC were cultured with 0–35 μM T3 in the presence of 10 ng/ml FGF for 72 h. Viable cells were estimated using the water-soluble tetrazolium salt (WST-1) assay. Values are presented as mean±S.D. (n=6).

chain of Toc [60]. Therefore, the greater antiangiogenic effect of T3 may be due, in part, to its effective incorporation into endothelial cells.

3.3. Antiangiogenic mechanism of T3

Several endothelial signaling pathways, particularly the PI3K/PDK/Akt pathway, are well known to be involved in tumor angiogenesis (Fig. 3A) [61,62]. In cancer patients, PI3K/PDK/Akt signaling is elevated in tumors and correlates with tumor progression [63]. PI3K is a lipid kinase that generates phosphatidylinositol (3,4,5)-trisphosphate (PIP3) as a second messenger, and PDK is activated by binding to PIP3 [64]. The activated PDK then phosphorylates and consequently activates Akt. Activated Akt has been shown to phosphorylate various proteins associated with endothelial cell survival and proliferation.

Considering the critical role of the PI3K/PDK/Akt signaling pathway in angiogenesis, we investigated whether the antiangiogenic effect of T3 is mediated through pathway modulation. In HUVEC culture, tube formation induced by a tumor-cell-cultured medium (CM) was accompanied by increased phosphorylation of PI3K/PDK/Akt pathway proteins such as PDK, Akt and PTEN [44]. Both tube formation and PI3K/PDK/Akt pathway protein phosphorylation were suppressed when δ-T3 was added to CM (Fig. 3B and C) [44]. We next investigated the effect of δ-T3 on signals related to PI3K/PDK/Akt, such as endothelial nitric oxide synthase (eNOS), glycogen synthase kinase 3 (GSK3) α/β and extracellular-signal-regulated kinase (ERK) 1/2, all of which are involved in cell proliferation and

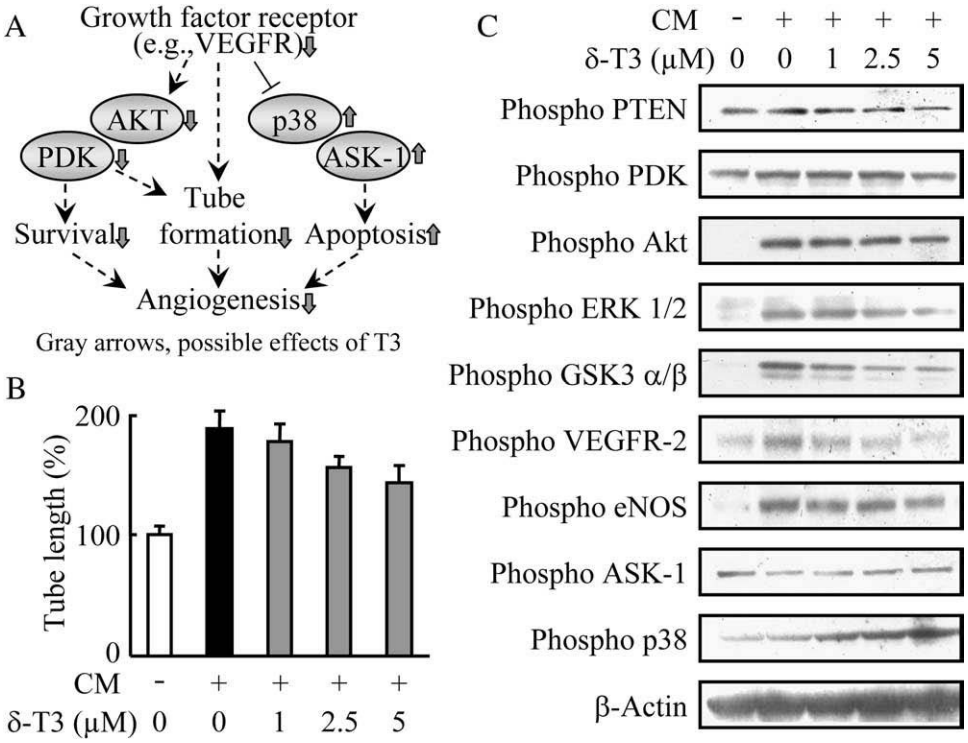

Fig. 3. Involvement of the PI3K/PDK/Akt pathway in tumor angiogenesis (A), and effects of δ-T3 on CM-induced HUVEC tube formation (B) and PI3K/PDK/Akt pathway protein phosphorylation (C). For tube formation assay (B), HUVEC were suspended in a medium containing δ-T3, and then mixed with CM. The cell suspension was incubated on a Matrigel plate for 18 h. Values are presented as mean±S.D. (n=6). For Western blot analysis (C), HUVEC were treated with 0–5 μM δ-T3 for 6 h, and then stimulated with CM for 10 min. Each blot is a representative example of data from three replicate experiments.

survival. Stimulation of HUVEC with CM resulted in activation of eNOS, GSK3 α/β and ERK 1/2, and the changes were reduced to basal (nonstimulated) levels by δ-T3 [44]. We also found that a relatively high dose of δ-T3 increased the phosphorylation of stress response proteins such as ASK-1 and p38 mitogen-activated protein kinase [44], which are involved in apoptosis induction in endothelial cells (Fig. 3A). These results suggested that PI3K/PDK/Akt signaling pathways mediate the antiangiogenic action of T3. Although suppression of the PI3K/PDK/Akt pathway is known to lead to apoptosis induction, as described above in cancer cells, the pathway may also contribute antiangiogenic effects at a lower dose level at which T3 does not exert obvious adverse effects on endothelial cells.

VEGF receptor 2 (VEGFR-2) acts as the key protein in growth factor signal transduction in endothelial cells [65,66], and regulation of VEGFR-2 activation would be a possible molecular target of antiangiogenic compounds [67]. We found that δ-T3 potently inhibits VEGF-induced VEGFR-2 phosphorylation in HUVEC [44], indicating that δ-T3 down-regulates upstream of the PI3K/PDK/Akt signaling pathway at the level of VEGFR-2. In contrast, α-Toc had no effect on the PI3K/PDK/Akt pathway and VEGFR-2 activation, which may account for its lower antiangiogenic activity. Further elucidation of detailed molecular mechanisms and precise molecular targeting of the antiangiogenic activity of T3 are subjects of ongoing investigations.

3.4. In vivo study of angiogenesis inhibition by T3

One of the problems encountered by angiogenesis researchers has been the difficulty of finding suitable methods for assessing the in vivo effects of angiogenic regulators [68]. The dorsal air sac (DAS) model is designed to be used in the examination of the in vivo effects of substances on the angiogenic response triggered by cancer cells [69]. The chick chorioallantoic membrane (CAM) assay is probably the most widely used in vivo assay for studying angiogenesis [70]. We chose these reliable methods for the evaluation of the antiangiogenic effect of T3 in this in vivo study.

In the DAS assay, increased neovascularization in tumor cell (human colon carcinoma, DLD-1)-implanted mice was suppressed by dietary supplementation of 10 mg/day T3-rich oil (equivalent to 4.4 mg/day T3) (Fig. 4) [43], indicating the in vivo antiangiogenic effects of T3. The calculated effective dose of T3 (150 mg/kg mouse/day) was lower than or similar to the reported dose associated with the anticancer activities of T3 in animal experiments [24]. We speculate that a substantial amount of orally administered T3 is absorbed from the intestinal tract and distributed to tissues surrounding the DLD-1 [71], where T3 inhibits vascularization of cells bearing growth factor receptors such as endothelial cells, smooth muscle cells and other cells responsible for neovascularization. However, in the DAS assay, there is little possibility that T3 also directly suppresses the proliferation of implanted tumor cells (the source of proangiogenic growth factors). The

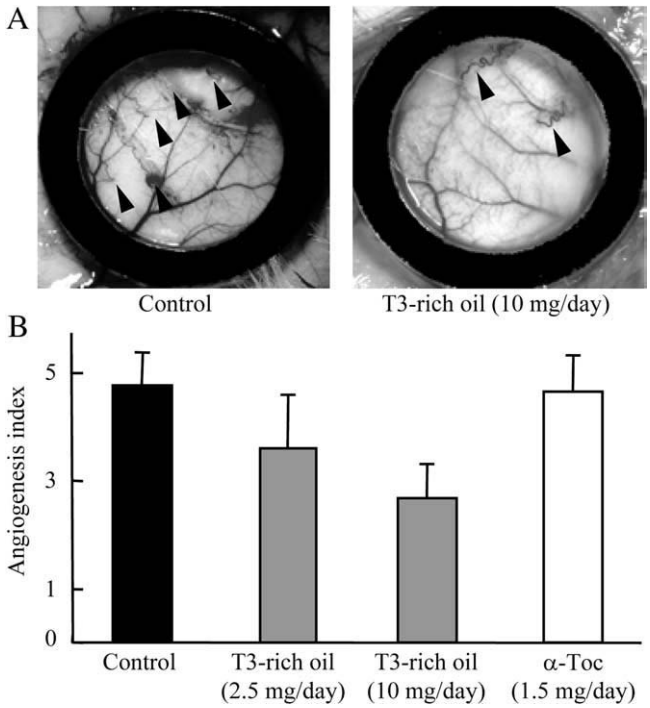

Fig. 4. Effects of T3-rich oil (Tocomin 50; Koyo Mercantile, Tokyo, Japan) on tumor-cell-induced angiogenesis in DLD-1-chamber-implanted mice fed Tocomin 50 (2.5 and 10 mg) or α-Toc (1.5 mg), by gavage, once a day for 5 days. Control mice received only the vehicle (vitamin-E-stripped corn oil). Implanted chambers were removed and photographed (A), and the number of new blood vessels was counted and scored as the angiogenesis index (B). Values are presented as mean±S.D. (n=10–12).

CAM assay was therefore performed for further evaluation of the in vivo antiangiogenic activity of T3. T3 treatment (500–1000 μg/egg) of CAM resulted in inhibition of angiogenic response (Fig. 5) [43], which occurred in the absence of any effect on embryo viability, suggesting little toxicity of T3 in vivo. As the chick embryo does not have established mature immune responses [70], the observed antiangiogenesis effects of T3 on CAM were presumably mediated by direct inhibition of endothelial cell function.

The above results indicate that T3 is an attractive candidate as an antiangiogenic agent. However, in future applications, it should be necessary to develop appropriate T3 treatment with minimal toxicity. For instance, the effect of T3 on physiological angiogenesis, such as wound healing, inflammation, ovulation, pregnancy, ischemia, hypertension or other diseases associated with bleeding [72], should be carefully investigated because dietary supplementation of vitamin E (Toc) has been reported to increase bleeding tendency [73].

4. Summary

In this review, the historical aspect and physiological activity (i.e., anticancer) of vitamin E were introduced, and

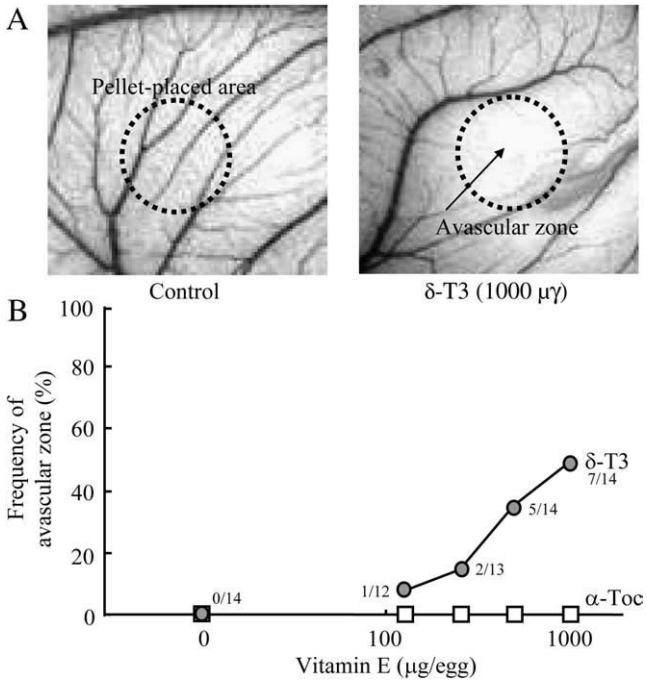

Fig. 5. Effects of T3 on the formation of new blood vessels in CAM. A pellet impregnated with δ-T3 (0–1000 μg), impregnated with α-Toc (0–1000 μg) or without vitamin E (control) was placed on the CAM and incubated for 2 days. The CAM was photographed (A), and angiogenic response was evaluated by measuring the avascular zone (B). The number of eggs with avascular zones over the total number of eggs (n=12–14).

current research on the angiogenesis-inhibitory effect of T3 and its related mechanism for cancer prevention was described. A series of experimental data from cell studies and animal models demonstrates that, among members of the vitamin E group, T3 performed potent antiangiogenic function in vitro and in vivo, and that the antiangiogenic effect of T3 was attributable to the regulation of growth factor signaling in endothelial cells. These findings indicate that T3 is a promising anticancer agent for minimizing tumor angiogenesis. The experimental data shown in this review warrant its testing in other models of cancer prevention, with a realistic prospect of its use for preventive and therapeutic approaches in humans.

Acknowledgments

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