Antioxidant and Anti-cancer Properties of Tocotrienols

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INTRODUCTION

Tocotrienols are fat soluble vitamins related to the family of tocopherols. The term vitamin E is now considered to be a generic name describing bioactivities of both tocopherol and tocotrienol derivatives. Structural difference between tocopherols and tocotrienols is in the presence of three double bonds in the carbon side chain of the tocotrienol molecule. While tocopherols are found most abundantly in the oils extracted from soyabean, cottonseed and sunflower seed, tocotrienols are found primarily in palm oil and the oil fractions of cereal grains such as wheat, barley and rice. The vitamin E of palm oil, unlike that of most other fats and oils, consists largely of tocotrienols with the remainder being α-tocopherol. Commercial quantities of tocotrienols are extracted from the distillates of palm oil and rice bran oil.

Tocotrienols have been the focus of increasing research interest in the last 5-10 years, not as secondary forms of vitamin E but as unique nutritional compounds. Scientific evidence has shown that in addition to being powerful biological antioxidants, these compounds may reduce cholesterol levels in people with hypercholesterolemia, may slow down the progression of atherosclerosis, and inhibit the proliferation and growth of human breast cancer cells.

This paper reviews some of the antioxidant and anti-cancer effects attributed to tocotrienols.

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ANTIOXIDANTS AND FREE RADICALS

Antioxidants are biochemical compounds which, as the name suggests prevent oxidation. Antioxidants represent a first line of defence against oxidative stress produced by the generation of free radicals and reactive oxygen species (ROS). If left unchecked, these compounds can cause considerable damage to tissues. Antioxidant nutrients have been in the media spotlight in recent years. The endless list of benefits attributed to antioxidants may suggest wonder drug status in the minds of some people. Even the sceptics have had to take notice of this special class of nutrients as their role in disease prevention has been demonstrated time and again.

What are Free Radicals?

A free radical is a highly charged particle formed as a result of oxidation. It is generated by the biochemical redox reactions that occur as part of normal cell metabolism, and by exposure to environmental factors such as UV light, cigarette smoke, environmental pollutants and gamma radiation.

A free radical has a missing or slightly damaged electron in its outer shell which it completes by grabbing onto or stealing from other molecules. The result of it grabbing or stealing electrons from other molecules is general damage and rusting. For example, free radicals cause cut apples to turn brown, steel to rust, white paint to darken, and rubber to harden. When a hose cracks or cement breaks, free radicals would most likely be behind the destruction.

The main free radical species and ROS which occur in the human body include:

- Hydroxy radical ($OH^{\bullet}$)
  - highly reactive radical which can attack all biological molecules.

- Superoxide radical ($O_2^{\bullet-}$)
  - less reactive radical which can travel in the blood and attack a number of biological targets.

- Nitric oxide radical ($NO^{\bullet}$)
  - acts on smooth muscle cells in vessel walls causing relaxation.

- Hydrogen peroxide (H_2O_2)
  - crosses cellular membranes easily and may cause expression of virus genes, *e.g.* HIV infected cells. This ROS has only a few cellular targets but can result in the production of hydroxyl radicals.

How do Free Radicals Harm Us?

The human body is constantly under attack from free radicals. In our body, free radicals either latch onto or take electrons from our healthy cells or genes. As a result, they damage cells, degrade collagen (which is responsible for the elasticity of skin), reprogramme our genes and even change the way cells replicate themselves. Once formed, free radicals attack cellular components, causing damage to lipids, proteins and DNA. This can initiate a chain of events which results in the onset of disease.

A typical external example of free radical damage to cells is old looking and wrinkly skin. Ultra violet rays from the sun dramatically increase free radical production in our body. Skin cancer can result from this over production of free radicals.

Experts call the general damaging effects of free radicals in the body as oxidative stress. Factors that increase the production of free radicals in the body and thus, oxidative stress, include smoking, drinking alcohol, emotional stress, pollution, pesticides, some drugs and toxic metals such as lead and cadmium.

Free radicals have been implicated in numerous diseases, including:

- Atherosclerosis
- Cancer
- Diabetes
- Respiratory disease
- Liver damage
- Rheumatoid arthritis
- Cataracts
- AIDS
- Inflammatory bowel disease
- Central nervous system disorders
- Parkinson's disease
- Motor neurone disease
- Conditions associated with premature birth

What Could be Done to Prevent the Damaging Effects of Free Radicals?

The best way to prevent the damaging effects of free radicals is to reduce their level in the body. This can be done by adopting a healthier life style including avoiding smoking, drinking alcohol in moderation, exercising regularly, avoiding mental and emotional stress, keeping the environment clean and pollution free, and staying away from toxic metals.

Another way to prevent the damaging effects of free radicals is to eat foods rich in antioxidants. Just as free radicals cause damage by oxidation, antioxidants reduce their damage by putting a stop to oxidation. Antioxidants do this by providing free radicals with the electrons they seek. This prevents them from stealing electrons from healthy cells.

ANTIOXIDANT PROPERTIES OF TOCOTRIENOLS

Vitamin E compounds are well known for their antioxidant property (Kamal-Eldin and Appelqvist, 1996). This property depends primarily on the phenolic group in the chromanol ring, rather than the side-chain (Niki *et al.*, 1985; Burton and Ingold, 1989). Tocotrienols, like tocopherols, are capable of scavenging and quenching free radicals. Their antioxidative activity, however, re-

sides mainly with their 'chain breaking' property which neutralizes peroxyl and alkoxyl radicals generated during lipid peroxidation (Kamal-Eldin and Appelqvist, 1996; Burton and Traber, 1990). Peroxidation of membrane lipids is known to modify and inactivate cellular components which can have damaging effects on crucial cellular factors leading to disease. In the case of LDL-lipids, peroxidation has emerged as the initiating step in the pathogenesis of atherosclerosis. Such modification of LDL causes recognition of the LDL-particle by the scavenger cell receptor present on macrophages. This pathway is unregulated and non-saturable, which consequently leads to massive deposition of cholesterol into these cells. This is typically associated with the formation of foam cells in the atherosclerotic lesions (Steinberg *et al,* 1989).

For many years, α-tocopherol was generally considered as the most potent antioxidant against lipid peroxidation in the vitamin E group (Burton *et al.*, 1983). However, considerable discrepancy has recently been found in its relative antioxidant effectiveness when compared to tocotrienols and the individual isomers. We have studied the *in vitro* effect of tocotrienol-rich fraction (TRF) on the peroxidation potential in rat liver mitochondria and microsomes, using ascorbate and Nicotine Adenine Dinucleotide Phosphate – reduced form (NADPH) induced systems. (Nesaretnam *et al.* 1993). Our results showed that TRF was able to inhibit lipid peroxidation in both mitochondria and microsomes in a dose dependent manner. Further experiments carried out showed that when compared to α-tocopherol, TRF showed better lipid peroxidaton potential (Kamat *et al.*, 1998) (*Figure 1*).

When the individual isomers were tested, α-tocotrienol was found to be a better antioxidant than α-tocopherol (Serbinova *et al.*, 1991; Suzuki *et al.*, 1993). Serbinova *et al.*, (1991) observed a remarkably higher antioxidant activity with tocotrienol against lipid peroxidation in rat liver liver microsomes than with α-tocopherol. Kamat and Devasagayam (1995) observed similar results in rat brain mitochondria and noted a

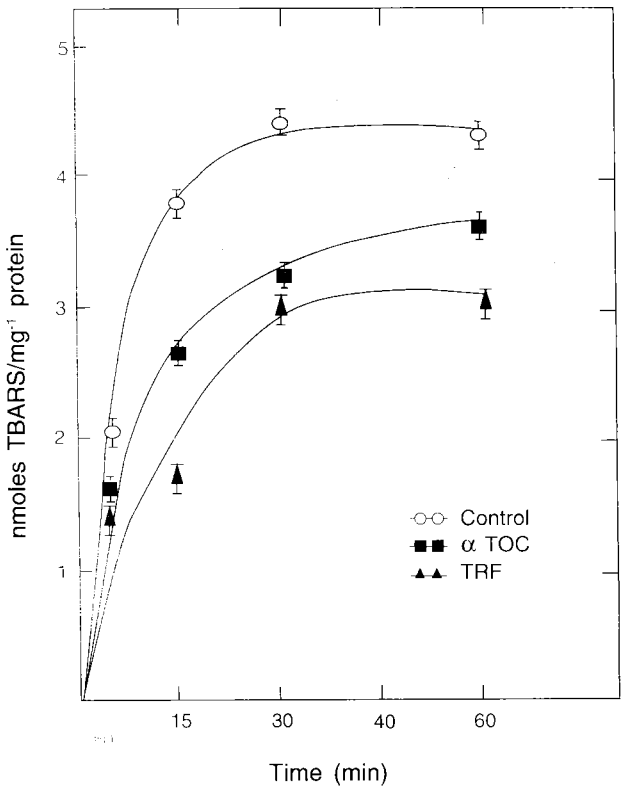

Figure 1. Ascobate-Fe2+ induced lipid peroxidation in rat liver microsmes and and its prevention by TRF and α-tocopherol as a function of time. Lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS). The concentration of α-tocopherol or TRF was 5 μM. Values are mean±SE from 5 experiments.

stronger effect with γ-tocotrienol. In an elaborate study, a number of mechanisms were shown to contribute to the higher antioxidant activity of tocotrienol compared to α-tocopherol (Serbinova *et al.*, 1996). They were:

- a more uniform distribution in the membrane lipid bilayer.
- a more efficient interaction of the chromanol ring with lipid radicals.
- a higher recycling efficiency from chromanoxyl radicals.

ANTI-CANCER PROPERTIES OF TOCOTRIENOLS

Evidence suggests that cancer development

occurs in two stages, initiation and promotion. Initiation involves a permanent, irreversible genetic change in the cells' DNA. This generally causes DNA strand breaks which can lie dormant in the cell but do not alone cause cancer. Promotion elicits the second stage of carcinogenesis which stimulates the initiated cell, transforming it to a cancerous cell. This process is reversible and may require continued stimulation to promote mutations. Many compounds have both initiating and promoting activities. However, free radicals are thought to act principally as promoting agents. Superoxide radicals and many peroxides are tumour promoters. Free radicals have been associated with gross chromosomal damage and inhibition of the biological natural repair systems. Free radicals can alter gene expression by the mobilization of calcium stores, which activates a variety of cellular kinases, phosphatases and transcription factors. Lipid peroxidation by free radicals has also been implicated as a causatory factor in cancer development.

Increased intake of antioxidants, either through the diet or, as dietary supplements, has been associated with a reduced incidence of cancer. Long term scientific studies have shown that low antioxidant levels are associated with increased incidence of certain cancers. Anti-carcinogenic and anti-promoter activity of some antioxidants is thought to offer some protection against cancer.

Our studies on breast cancer looked at the protective role palm oil plays in mammary carcinogenesis and compared the positive effect conferred by palm oil and linked it to the presence of vitamin E (Nesaretnam *et al.*, 1992). Further work was then carried out *in vitro* on the growth of estrogen receptor positive and receptor negative human breast cancer cells using both TRF as well as the individual tocotrienols.

Breast cancer was chosen as a subject for investigation as it is the leading cause of death amongst women. In Malaysia, breast cancer represented 9% of total cancer incidence in 1993. While it was estimated that 1800 women developed breast cancer annually, the number had increased substantially by 1996. Three thousand five hundred new breast cancer cases were reported in the whole country. Two hundred and twenty women died of the disease in the same year (Annual Report, Ministry of Health, 1996). More attention should be focused on measures that can be taken to lower the risk of women developing breast cancer. It is therefore reassuring to know that the development of more effective biological therapies for breast cancer is under intense investigation. Investigation of fat soluble vitamins (A,D,E and K) for anti-cancer properties have shown them to possess anti-proliferative effects.

The tumour protective effect of tocotrienols has also been studied *in vitro* on human breast cancer cell lines. The first set of experiments showed that TRF inhibited the incorporation of [^{3}H] thymidine into these cells by 50% at a concentration of 180 μg ml^{-1} of TRF. In comparison, α-tocopherol did not significantly inhibit growth at concentrations up to 500 μg ml^{-1}. Subsequent experiments showed growth of the cells was inhibited by 50% in the presence of 180 μg ml^{-1} whereas no growth inhibition was observed with a concentration of 500 μg ml^{-1} of α-tocopherol (Nesaretnam *et al.*, 1995).

These results indicate that the inhibition of proliferation and growth of the cells by TRF was probably due to tocotrienols. To confirm this, oestrogen-receptor negative human breast cancer cells were incubated with varying concentrations of individual tocotrienols. Experiments on the incorporation of [^{3}H] thymidine gave an LD_{50} of 28 μg ml^{-1} for γ-tocotrienol and 90 μg ml^{-1} for both α- and δ-tocotrienols (Carroll *et al.*, 1995). Further experiments carried out on oestrogen receptor positive human breast cancer cells showed TRF inhibited proliferation of the cells with maximal effect at 8 μg ml^{-1}. In contrast, α-tocopherol had no effect (*Figure 2*). The individual tocotrienol fractions (γ and δ) showed even greater inhibitory effects at lower concentrations from 1-6 μg ml^{-1} with γ-tocotrienol having the greatest effect (*Figure 3*). In addition to their

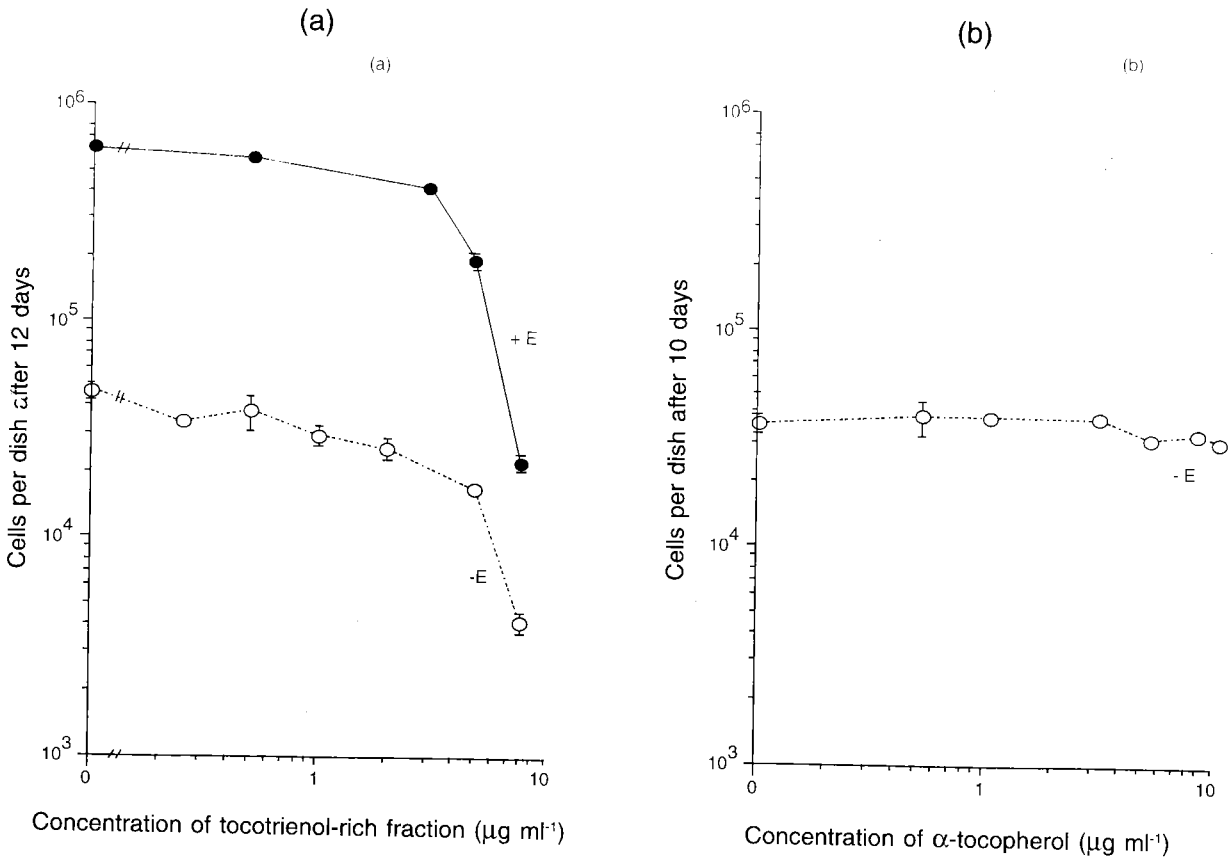

Figure 2. Effect of the tocotrienol-rich fraction (A) or α-tocopherol (B) on the growth of oestrogen receptor positive MCF-7 human breast cancer cells. Cells were grown in 24 well dishes in RPMI 1640 medium without phenol red but containing 5% DCFCS. (a) Cells were grown for 12 days with increasing concentrations of TRF in either the absence (...o...) or presence (-•-) of 10-^{8}M oestradiol. (b) Cells were grown for 10 days with increasing concentrations of α-tocopherol in the absence of oestradiol (...o...).

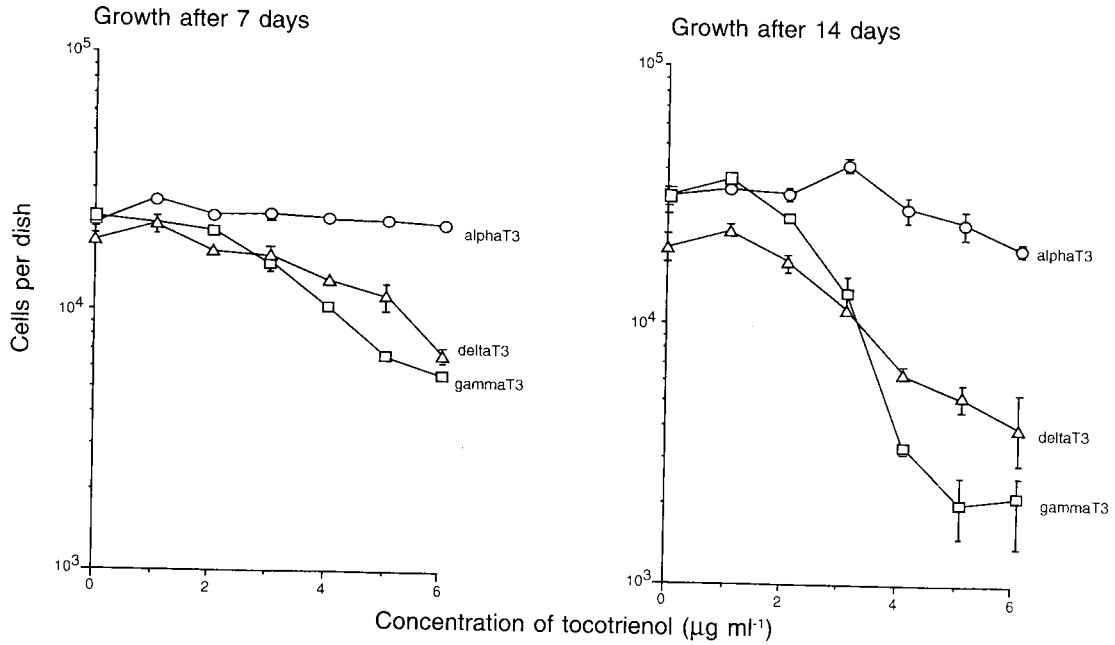

Figure 3. Effect of individual tocotrienols on the growth of oestrogen responsive MCF-7 human breast cancer cells. Cell were grown for seven or 14 days in 24 well dishes in RPMI 1640 medium without phenol red but containing 5% DCFCS and increasing concentrations of α-T3, γ-T3 or δ-T3.

anti-proliferative effect, γ- and δ-tocotrienols also resulted in decreased expression of insulin-like growth factor binding proteins (IGFBP)-2 and -4. Recent evidence suggests IGF binding proteins can influence interactions between IGFs and their receptors. As such, IGFBPs play an important role in mediating IGF induced growth of breast cancer.

The expression of pS2, a well established oestrogen regulated gene, was also determined. Northern blotting of pS2 mRNA from the breast cancer cells (*Figure 4*) demonstrated TRF did not inhibit expression of the gene, indicating the mechanism of inhibition by tocotrienols was different from that of an oestrogen antagonist such as tamoxifen which is widely used in breast cancer therapy (Nesaretnam *et al.*, 1998). Similar results were obtained by Guthrie *et al.* (1997) on oestrogen receptor positive human breast cancer cells. They showed that tocotrienols inhibited proliferation of the cells as measured by [^{3}H]thymidine incorporation. The LD_{50} for TRF, α-tocopherol, α-,γ- and δ-tocotrienols were 4, 125, 6, 2, and 2 μg ml^{-1} respectively.

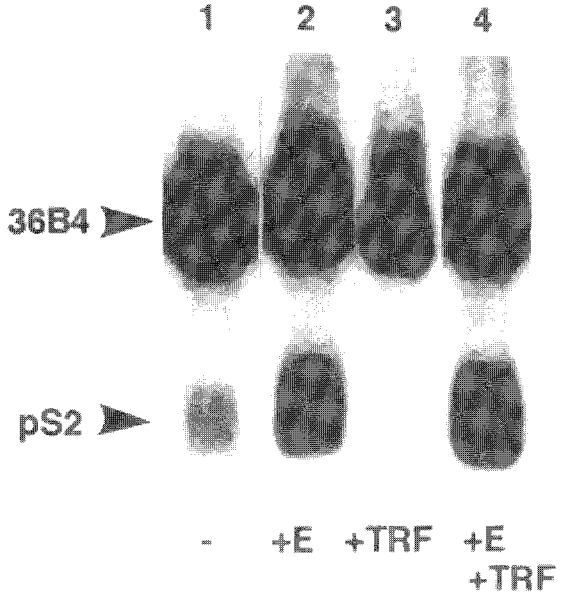

Figure 4. Effect of the tocotrienol-rich fraction (TRF) of palm oil on expression of pS2 mRNA in MCF-7 human breast cancer cells. Northern blot of whole RNA from cells grown for six days in monolayer culture with DCFCS alone (-) (track 1), with 10^{-8}M oestradiol (+E) (track 2), with 8 μg ml^{-1} TRF (+TRF) (track 3) or with both 10^{-8}M oestradiol and 8 μg ml^{-1} TRF (+E+TRF) (track 4). The blot was probed for expression of the oestrogen regulated pS2 mRNA and also a control for any equal RNA loadings.

Other evidence that vitamin E from palm oil may possess anti-cancer properties has come from experiments with transplantable tumours in mice (Komiyama *et al.*, 1989). When injected intraperitoneally into mice, α- and γ-tocotrienols prolonged the life of mice bearing Ehrlich sarcoma, sarcoma 180, or IMC carcinoma. Gama-tocotrienol was more effective than α-tocotrienol in each case and α-tocopherol was least effective. In addition to studies on experimental animals, Komiyama and Yamaoka (1989) showed the growth inhibition of human and mouse tumour cells (H69, HeLa and P388) when the cells were exposed to tocotrienols for 72 hr *in vitro*. Survival of mice receiving transplantable tumour cells was also increased if they were fed tocotrienols in a dose responsive way (Kato *et al.*, 1985). Tocotrienols have also caused a delay in the onset of subcutaneous lymphoma by 2-4 weeks in HRS/J hairless mice, a strain genetically susceptible to subcutaneous lymphoma (Tan, 1992).

The precise mechanism for the anti-proliferative property of tocotrienols is uncertain, but may lie in its prenylated side-chain involved in the production of isoprenoid intermediates from the mevalonate biosynthetic pathway (Crowell *et al.*, 1991; Elson and Yu, 1994). These intermediates are thought to be involved in the prenylation of several signal transduction proteins including the Ras protein, essential for normal cell growth.

CONCLUSION

Collectively known as vitamin E, tocotrienols are identical in structure to tocopherols except for the degree of saturation in their side chain. The prenyl side-chain of tocotrienol has been postulated to be responsible for the differential membrane distribution

and metabolism of tocotrienols when compared with tocopherols. Future investigations need to examine the molecular mechanism of action of tocotrienols in order to achieve a more comprehensive understanding of their complex, but beneficial effects on cancer.

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