Tocotrienols: Constitutional Effects in Aging and Disease[1]

Sebastian Schaffer,[2] Walter E. Müller, and Gunter P. Eckert

Institute of Pharmacology (ZAFES Member), Biocenter Niederursel, University of Frankfurt, Frankfurt am Main, Germany

ABSTRACT Tocotrienols, a class of vitamin E analogs, modulate several mechanisms associated with the aging process and aging-related diseases. Most studies compare the activities of tocotrienols with those of tocopherols ("classical vitamin E"). However, some biological effects were found to be unique for tocotrienols. Although the absorption mechanisms are essentially the same for all vitamin E analogs, tocotrienols are degraded to a greater extent than tocopherols. The levels of tocotrienols in the plasma of animals and humans were estimated to reach low micromolar concentrations. One hallmark in the origin of disease and aging is the overproduction of reactive oxygen species (ROS). Tocotrienols possess excellent antioxidant activity in vitro and have been suggested to suppress ROS production more efficiently than tocopherols. In addition, tocotrienols show promising nonantioxidant activities in various in vitro and in vivo models. Most notable are the interactions of tocotrienols with the mevalonate pathway leading to the lowering of cholesterol levels, the prevention of cell adhesion to endothelial cells, and the suppression of tumor cell growth. Furthermore, glutamate-induced neurotoxicity is suppressed in the presence of tocotrienols. This review summarizes the main antioxidant and nonantioxidant effects of tocotrienols and assesses their potential as health-maintaining compounds. J. Nutr. 135: 151–154, 2005.

KEY WORDS: • tocotrienols • antioxidants • cholesterol • cancer • neurotoxicity

Natural vitamin E includes 2 groups of closely related fat-soluble compounds, the tocotrienols (TCTs)[3] and tocopherols (TCPs), each with the 4 analogs, α, β, γ, and δ (**Fig. 1**). All vitamin E analogs possess a 6-membered, aromatic chromanol ring structure and a side chain. The TCPs have a phytol chain, whereas the TCTs have an unsaturated side chain with double bonds at the 3′, 7′, and 11′ positions of the hydrocarbon tail. Based on its lipophilicity, vitamin E is considered to be the major chain-breaking antioxidant preventing the propagation of oxidative stress, especially in biological membranes (1). Recent data suggest that a common mechanism based on oxidative stress emanating from an overproduction of reactive oxygen species (ROS) contributes to the aging process and aging-related diseases in many or even all species (2). Furthermore, the combination of a Western lifestyle, a nonbalanced diet, and increasing mean life expectancy is responsible for the frequent occurrence of cardiovascular, malignant and neurodegenerative diseases. On the basis of epidemiologic and retrospective studies, supplementation with antioxidants such as TCPs is considered to be a nutrition-based strategy to prevent diseases and to support healthy aging. However, findings from controlled clinical trials failed to show a strong effect of TCP intake on Alzheimer's and cardiovascular disease progression (3,4). Recently, TCTs have gained increasing scientific interest due to their eminent antioxidant effects and an nonantioxidant activity profile that differs somewhat from that of TCPs. TCTs are found in abundance in plant foods such as rice bran or palm oil (5). This article reviews the potential effect of TCTs on the aging process as well as the emergence and progression of aging-related diseases.

Tocotrienol Bioavailability. There is still much controversy about the absorption, retention, and metabolism of TCTs (**Fig. 2**). Cell culture studies suggest that TCTs and TCPs are metabolized similarly, i.e., by ω-oxidation followed by β-oxidation of the side chain, although TCTs were found to be degraded to a larger extent than TCPs (6). Recently, Yap et al. (7) investigated in humans the bioavailability and pharmacokinetics of α-, γ-, and δ-TCT in different food states. The plasma levels of all 3 TCTs increased markedly with food intake. Thereafter, TCT concentrations in the plasma dropped rapidly initially followed by a more gradual decline. TCT plasma levels were reported to reach 1 μmol/L in humans and between 3 and 20 μmol/L in various animal species (8–11). TCTs were found in various tissues of rats, especially adipose tissues, skin, and heart, after oral application, suggesting that TCTs are absorbed and distributed in vivo (12,13). Moreover, oral supplementation of female rats produced a moderate accumulation of TCTs in the brains and an even significantly higher TCT concentration in the brain of rat pups (14).

Antioxidant Activity of Tocotrienols. The antioxidant potencies of TCTs and TCPs were compared in a variety of extensive in vitro experiments, although with conflicting results. TCTs showed a >60% lower half-maximal antioxidant efficiency concentration in liposomes than TCPs (15,16). This superior antioxidant activity of TCTs is thought to be related to a more uniform distribution within membranes, a disordering effect on the membrane lipids, and a higher recycling efficiency from chromoxyl radicals (17). In contrast, a recent study (18) showed only marginal, if any, differences between the antioxidant activity of TCTs and TCPs in cell-free and liposome test systems. However, given that only one concentration of TCTs and TCPs was compared, the importance of

[1] Manuscript received 27 September 2004.
[2] To whom correspondence should be addressed. E-mail: S.Schaffer@em.uni-frankfurt.de.
[3] Abbreviations used: α-TTP, α-tocopherol transfer protein; CD, cardiovascular disease; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; GSH, glutathione; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; HUVEC, human umbilical vein endothelial cells; 12-LOX, 12-lipoxygenase; ROS, reactive oxygen species; TCP, tocopherol; TCT, tocotrienol; VCAM-1, vascular cellular adhesion molecule-1.

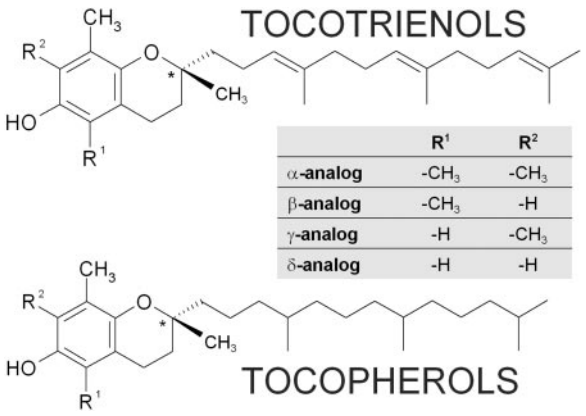

FIGURE 1 Structure of tocotrienols and tocopherols.

the latter study is limited. Also, when investigated under more elaborated experimental settings, TCTs protect cells to a greater extent than TCPs or vitamin C against ROS-mediated lipid peroxidation or erythrocyte hemolysis (19,20). Similarly, in *Caenorhabditis elegans* exposed to UV-B light, pre- and postadministration of TCTs lowered protein carbonylation and extended mean life span. In contrast, α-TCP supplementation did not have any significant effects (21). In addition, TCTs recently displayed high antioxidant activity in feeding experiments in rats and humans (10,22).

Tocotrienols Modulate Cardiovascular Disease Risk Parameters. Cardiovascular disease (CD) remains one of the most important causes of morbidity and mortality, especially in men and women > 60 y old. The lowering of cholesterol levels was shown to reduce the risk of CD. Statins reduce cholesterol by competitively inhibiting the rate-limiting enzyme of cholesterol synthesis 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, whereas TCTs produce this effect post-transcriptionally by increasing HMG-CoA reductase degradation as well as decreasing the efficiency of the translation of HMG-CoA reductase mRNA (23). The in vitro cholesterol-lowering effect of TCTs or TCT-rich fractions was also shown in various animal models (9,24). Data for humans, however, are less consistent. In placebo-controlled, double-blind studies, Mensink et al. (25) and Mustad et al. (11) did not find any favorable effects of different TCT combinations on serum lipoprotein profile in hypercholesterolemic patients after 4- (200 mg TCT/d) and 6-wk (160 mg TCT/d) supplementation periods. The outcome of these studies is in contrast to publications from Qureshi et al. (26,27), which reported significant cholesterol-reducing effects of TCTs and TCT-rich fractions in animals and humans. The contradictory effects of TCTs on cholesterol levels were suggested to be due to differences in dietary cholesterol and energy intake of study subjects. Furthermore, α-TCP concentrations > 20% in TCT-rich supplements might attenuate the cholesterol-lowering potential of TCTs (27), an explanation that was questioned, however, in a review by Kerckhoffs et al. (28). Recently, Qureshi et al. (29) confirmed their earlier results by reporting a dose-dependent suppression of serum cholesterol by a TCT-rich (25–200 mg/d) fraction of rice bran in hypercholesterolemic humans following the American Heart Association Step-1 diet. Similar effects were found in rats with experimental hypercholesterolemia that were fed a TCT-rich fraction for 6 mo. Reduced enzymatic activity and protein mass of HMG-CoA reductase subsequently led to a significant reduction in plasma cholesterol (30).

Adhesion molecules are also involved in atherogenesis by mediating cell adhesion to endothelial cells. Theriault et al. (31) reported that α-TCT markedly inhibited not only the surface expression of cell adhesion molecules [vascular cellular adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1, E-selectin] in human umbilical vein endothelial cells (HUVEC) but also monocytic cell adhesion of THP-1 cells. Both effects were more pronounced for α-TCT than for α-TCP. An in vitro study by Noguchi et al. (32) found that α-TCT affected VCAM-1 expression similarly at 90% lower concentrations than that of α-TCP (e.g., 2.5 vs. 25 μmol/L, respectively). The same difference in efficiency was observed in THP-1 adhesion to HUVEC. The superior inhibitory effect of α-TCT has been linked to its 10-fold higher intracellular accumulation. Moreover, in a direct comparison of various TCT analogs, δ-TCT displayed the highest efficiency toward the prevention of adhesion molecule expression and monocytic cell adhesion. Changes in protein prenylation, via inter-

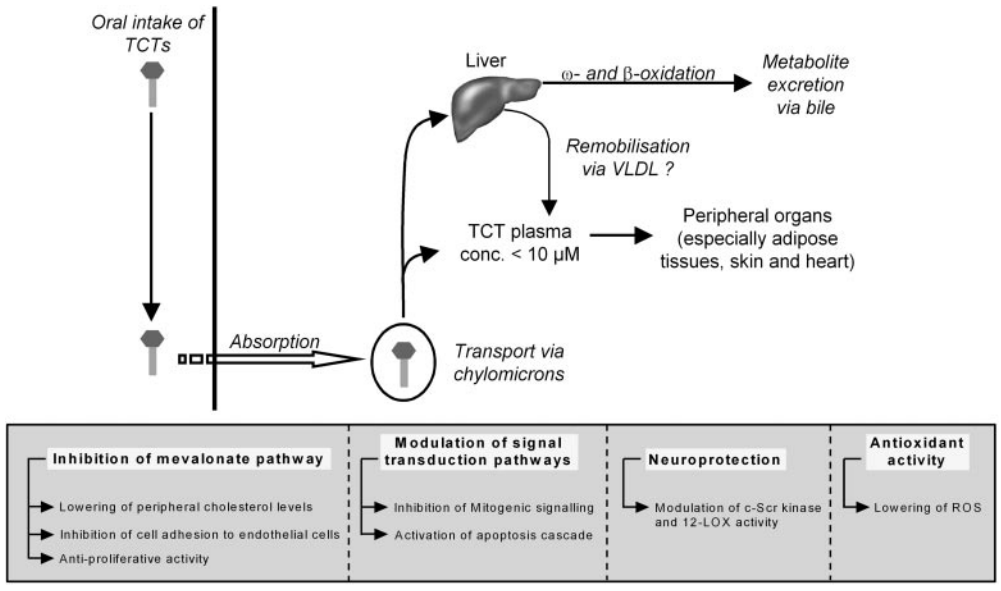

FIGURE 2 Overview on the absorption and metabolism of tocotrienols and their proposed effects in vitro and in vivo.

ference with the HMG-CoA reductase-controlled mevalonate pathway, might be responsible for the observed effects of TCTs (33).

In hypertension, another important risk factor for the development of CD, endothelium-dependent vascular relaxation is impaired. Reduction of eNOS activity and increased removal of NO in blood vessels were postulated to mediate the observed impairment of vascular relaxation. In spontaneously hypertensive rats, Newaz et al. (22) observed a significant reduction of systolic blood pressure after a 3-mo intervention with γ-TCT (15 mg/kg diet). Additionally, γ-TCT treatment improved eNOS activity, leading to a significant negative correlation with systolic blood pressure.

Antiproliferative Activity of Tocotrienols. Cancer, still one of the leading causes of death, is tightly linked to the aging-process. Earlier studies showed that high dietary intake of vitamin E contributes to the suppression of tumorigenesis. Subsequently, in vitro experiments were performed to determine the antiproliferative efficiency of various vitamin E analogs and to elucidate mechanisms facilitating cell-growth inhibition. Long-term incubation with several TCTs or TCT-rich fractions in the low micromolar range inhibited growth of preneoplastic, neoplastic, and highly malignant mouse mammary epithelial cells by 50%. In contrast, treatment with 0–120 μmol/L α- and γ-TCP had no effect on cell proliferation. Furthermore, TCTs were preferentially accumulated by these cells and led to higher rates of DNA fragmentation (34). A study by Sylvester et al. (35) indicated that the effects of TCTs on cell proliferation are due to an inhibitory activity in early post-epidermal growth factor (EGF) receptor events involved in cAMP production downstream from EGF-dependent mitogenic signaling, rather than a reduction in EGF-receptor mitogenic responsiveness. The authors suggested that TCTs specifically inhibit cAMP-dependent protein kinase mitogenic signaling, in particular the phosphoinositide 3-kinase/Akt pathway. Recently, Takahashi and Loo (36) showed in human breast cancer cells that incubation with γ-TCT induced a significant reduction in mitochondrial membrane potential, an early step during the initiation of apoptosis. Moreover, cytochrome *c* was released from the mitochondria into the cytosol after γ-TCT treatment. Cytochrome *c* contributes to the formation of the apoptosome, a complex associated with the activation of the caspase-controlled cell death pathway. Despite the observed cytochrome *c* release, no changes in the Bax:Bcl-2 ratio or caspase-3 activity occurred. Hence, the apoptotic effect of γ-TCT contrasts with that of some other phytochemicals. A further insight in TCT-mediated anti-proliferative activity is provided in a recent review by Mo and Elson (37) who focused on HMG-CoA reductase activity, which is elevated and deregulated in tumor cells. The HMG-CoA reductase-controlled mevalonate pathway is responsible for the supply of downstream metabolites essential for the function of growth factor receptors. Despite a resistance to sterol feedback regulation, tumor cell HMG-CoA reductase remains sensitive to isoprenoid-mediated downregulation. Farnesol acts as a mevalonate-derived mediator of HMG-CoA reductase degradation. Hence, the fact that TCTs are farnesol homologs might explain their potential in preventing cell proliferation by modulating HMG-CoA reductase degradation and translation of its mRNA.

Tocotrienols Are Neuroprotective In Vitro. Oxidative stress-induced programmed cell death, oxytosis, is one hallmark of neurodegenerative diseases. Elevated concentrations of extracellular glutamate inhibit the uptake of cystine and thus induce oxidative stress. Studies in HT4 hippocampal neuronal cells showed that activation of c-Src kinase and phosphorylation of extracellular signal-regulated kinase, both central events in glutamate-induced cell death, are blocked by nanomolar concentrations of α-TCT (38). For α-TCP, however, no such effects were found. Khanna et al. (39) further explored the underlying mechanisms of TCT-mediated neuroprotection. Lowering the levels of intracellular GSH triggers the activation of 12-lipoxygenase (12-LOX), which facilitates cell death by the production of peroxides and calcium influx. Primary cortical neurons were resistant to glutamate-induced cell death in the presence of physiologically relevant concentrations of α-TCT. Additional experiments indicated that α-TCT controls 12-LOX activity by hindering the access of arachidonic acid to the catalytic site of 12-LOX (39). Comparative studies with TCPs, however, are missing. Both studies suggest that α-TCT prevents glutamate-induced neurotoxicity by modulating the activity of important proteins involved in cell death signal transduction.

Concluding Remarks. The antioxidant potential of TCTs has been the main focus of scientific interest in recent years. In vitro experiments repeatedly showed higher radical scavenging efficiency for TCTs compared with TCPs. However, this observation is challenged by data from recent studies. Furthermore, only a limited number of studies investigated the antioxidant effects of TCTs in vivo. Although promising, these data do not yet provide a sufficient basis for a dietary recommendation of TCTs as antioxidants with superior radical scavenging power. More and better-designed in vivo studies in animals and especially humans are warranted not only to address the question of the ROS-scavenging potential of TCTs compared with other antioxidants, but also to estimate possible synergistic or opposing interactions of TCTs with other elements of the antioxidant network. The nonantioxidant effects of TCTs address several health aspects associated with the aging process as well as aging-related disease. Here, the interference of TCTs with the mevalonate pathway is of particular interest because its inhibition subsequently leads to alleviated levels of peripheral cholesterol, inhibition of cell adhesion to endothelial cells, and suppression of tumor cell growth. The in vivo cholesterol-lowering effects of TCTs, however, remain inconclusive; we suggest that studies considering important parameters such as defined compositions of TCT supplements, patient selection, and accompanying dietary regimen will provide insight into the nature of the cholesterol-lowering potential of TCTs. Similarly, the observed antiproliferative and neuroprotective activity of TCTs in vitro must be confirmed in vivo.

LITERATURE CITED

1. Ricciarelli, R., Zingg, J. M. & Azzi, A. (2001) Vitamin E: protective role of a Janus molecule. FASEB J. 15: 2314–2325.
2. Golden, T. R., Hinerfeld, D. A. & Melov, S. (2002) Oxidative stress and aging: beyond correlation. Aging Cell 1: 117–123.
3. McDaniel, M. A., Maier, S. F. & Einstein, G. O. (2003) "Brain-specific" nutrients: a memory cure? Nutrition 19: 957–975.
4. Vivekananthan, D. P., Penn, M. S., Sapp, S. K., Hsu, A. & Topol, E. J. (2003) Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet 361: 2017–2023.
5. Theriault, A., Chao, J. T., Wang, Q., Gapor, A. & Adeli, K. (1999) Tocotrienol: a review of its therapeutic potential. Clin. Biochem. 32: 309–319.
6. Birringer, M., Pfluger, P., Kluth, D., Landes, N. & Brigelius-Flohe, R. (2002) Identities and differences in the metabolism of tocotrienols and tocopherols in HepG2 cells. J. Nutr. 132: 3113–3118.
7. Yap, S. P., Yuen, K. H. & Wong, J. W. (2001) Pharmacokinetics and bioavailability of alpha-, gamma- and delta-tocotrienols under different food status. J. Pharm. Pharmacol. 53: 67–71.
8. Yap, S. P., Yuen, K. H. & Lim, A. B. (2003) Influence of route of

administration on the absorption and disposition of alpha-, gamma- and delta-tocotrienols in rats. J. Pharm. Pharmacol. 55: 53–58.

9. Raederstorff, D., Elste, V., Aebischer, C. & Weber, P. (2002) Effect of either gamma-tocotrienol or a tocotrienol mixture on the plasma lipid profile in hamsters. Ann. Nutr. Metab. 46: 17–23.

10. O'Byrne, D., Grundy, S., Packer, L., Devaraj, S., Baldenius, K., Hoppe, P. P., Kraemer, K., Jialal, I. & Traber, M. G. (2000) Studies of LDL oxidation following alpha-, gamma-, or delta-tocotrienyl acetate supplementation of hypercholesterolemic humans. Free Radic. Biol. Med. 29: 834–845.

11. Mustad, V. A., Smith, C. A., Ruey, P. P., Edens, N. K. & DeMichele, S. J. (2002) Supplementation with 3 compositionally different tocotrienol supplements does not improve cardiovascular disease risk factors in men and women with hypercholesterolemia. Am. J. Clin. Nutr. 76: 1237–1243.

12. Ikeda, S., Tohyama, T., Yoshimura, H., Hamamura, K., Abe, K. & Yamashita, K. (2003) Dietary α-tocopherol decreases α-tocotrienol but not γ-tocotrienol concentration in rats. J. Nutr. 133: 428–434.

13. Okabe, M., Oji, M., Ikeda, I., Tachibana, H. & Yamada, K. (2002) Tocotrienol levels in various tissues of Sprague-Dawley rats after intragastric administration of tocotrienols. Biosci. Biotechnol. Biochem. 66: 1768–1771.

14. Roy, S., Lado, B. H., Khanna, S. & Sen, C. K. (2002) Vitamin E sensitive genes in the developing rat fetal brain: a high-density oligonucleotide microarray analysis. FEBS Lett. 530: 17–23.

15. Suzuki, Y. J., Tsuchiya, M., Wassall, S. R., Choo, Y. M., Govil, G., Kagan, V. E. & Packer, L. (1993) Structural and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry 32: 10692–10699.

16. Qureshi, A. A., Mo, H., Packer, L. & Peterson, D. M. (2000) Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. J. Agric. Food Chem. 48: 3130–3140.

17. Serbinova, E., Kagan, V., Han, D. & Packer, L. (1991) Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. Free Radic. Biol. Med. 10: 263–275.

18. Yoshida, Y., Niki, E. & Noguchi, N. (2003) Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chem. Phys. Lipids 123: 63–75.

19. Mutalib, M.S.A., Khaza'ai, H. & Wahle, K.W.J. (2003) Palm-tocotrienol rich fraction (TRF) is a more effective inhibitor of LDL oxidation and endothelial cell lipid peroxidation than alpha-tocopherol in vitro. Food Res. Int. 36: 405–413.

20. Begum, A. N. & Terao, J. (2002) Protective effect of alpha-tocotrienol against free radical-induced impairment of erythrocyte deformability. Biosci. Biotechnol. Biochem. 66: 398–403.

21. Adachi, H. & Ishii, N. (2000) Effects of tocotrienols on life span and protein carbonylation in Caenorhabditis elegans. J. Gerontol. A Biol. Sci. Med. Sci. 55: B280–B285.

22. Newaz, M. A., Yousefipour, Z., Nawal, N. & Adeeb, N. (2003) Nitric oxide synthase activity in blood vessels of spontaneously hypertensive rats: antioxidant protection by gamma-tocotrienol. J. Physiol. Pharmacol. 54: 319–327.

23. Cicero, A. F. & Gaddi, A. (2001) Rice bran oil and gamma-oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phytother. Res. 15: 277–289.

24. Black, T. M., Wang, P., Maeda, N. & Coleman, R. A. (2000) Palm tocotrienols protect ApoE +/- mice from diet-induced atheroma formation. J. Nutr. 130: 2420–2426.

25. Mensink, R. P., van Houwelingen, A. C., Kromhout, D. & Hornstra, G. (1999) A vitamin E concentrate rich in tocotrienols had no effect on serum lipids, lipoproteins, or platelet function in men with mildly elevated serum lipid concentrations. Am. J. Clin. Nutr. 69: 213–219.

26. Qureshi, A. A., Peterson, D. M., Hasler-Rapacz, J. O. & Rapacz, J. (2001) Novel tocotrienols of rice bran suppress cholesterogenesis in hereditary hypercholesterolemic swine. J. Nutr. 131: 223–230.

27. Qureshi, A. A., Sami, S. A., Salser, W. A. & Khan, F. A. (2001) Synergistic effect of tocotrienol-rich fraction (TRF(25)) of rice bran and lovastatin on lipid parameters in hypercholesterolemic humans. J. Nutr. Biochem. 12: 318–329.

28. Kerckhoffs, D. A., Brouns, F., Hornstra, G. & Mensink, R. P. (2002) Effects on the human serum lipoprotein profile of β-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. J. Nutr. 132: 2494–2505.

29. Qureshi, A. A., Sami, S. A., Salser, W. A. & Khan, F. A. (2002) Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. Atherosclerosis 161: 199–207.

30. Iqbal, J., Minhajuddin, M. & Beg, Z. H. (2003) Suppression of 7,12-dimethylbenz[α]anthracene-induced carcinogenesis and hypercholesterolaemia in rats by tocotrienol-rich fraction isolated from rice bran oil. Eur. J. Cancer Prev. 12: 447–453.

31. Theriault, A., Chao, J. T., Gapor, A., Chao, J. T. & Gapor, A. (2002) Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecules and adhesion to monocytes. Atherosclerosis 160: 21–30.

32. Noguchi, N., Hanyu, R., Nonaka, A., Okimoto, Y. & Kodama, T. (2003) Inhibition of THP-1 cell adhesion to endothelial cells by alpha-tocopherol and alpha-tocotrienol is dependent on intracellular concentration of the antioxidants. Free Radic. Biol. Med. 34: 1614–1620.

33. Chao, J. T., Gapor, A. & Theriault, A. (2002) Inhibitory effect of delta-tocotrienol, a HMG CoA reductase inhibitor, on monocyte-endothelial cell adhesion. J. Nutr. Sci. Vitaminol. 48: 332–337.

34. McIntyre, B. S., Briski, K. P., Gapor, A. & Sylvester, P. W. (2000) Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. Proc. Soc. Exp. Biol. Med. 224: 292–301.

35. Sylvester, P. W., Nachnani, A., Shah, S. & Briski, K. P. (2002) Role of GTP-binding proteins in reversing the antiproliferative effects of tocotrienols in preneoplastic mammary epithelial cells. Asia Pac. J. Clin. Nutr. 11 (suppl. 7): S452–S459.

36. Takahashi, K. & Loo, G. (2004) Disruption of mitochondria during tocotrienol-induced apoptosis in MDA-MB-231 human breast cancer cells. Biochem. Pharmacol. 67: 315–324.

37. Mo, H. & Elson, C. E. (2004) Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention. Exp. Biol. Med. 229: 567–585.

38. Sen, C. K., Khanna, S., Roy, S. & Packer, L. (2000) Molecular basis of vitamin E action. Tocotrienol potently inhibits glutamate-induced pp60(c-Src) kinase activation and death of HT4 neuronal cells. J. Biol. Chem. 275: 13049–13055.

39. Khanna, S., Roy, S., Ryu, H., Bahadduri, P., Swaan, P. W., Ratan, R. R. & Sen, C. K. (2003) Molecular basis of vitamin E action: tocotrienol modulates 12-lipoxygenase, a key mediator of glutamate-induced neurodegeneration. J. Biol. Chem. 278: 43508–43515.