

Tocotrienols: The lesser known form of natural vitamin E

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A recent and growing body of research has shown that members of this vitamin E family possess unique biologic functions. Tocotrienols have garnered much of this recent attention, and in particular α -tocotrienol has been shown to be the most potent neuroprotective form of vitamin E. Protection exclusively mediated through tocotrienols has been attributed to many mechanisms including inhibition of 12-LOX, c-Src, PLA₂ and through up-regulation of MRP1. Further, tocotrienols have recently been shown to induce arteriogenesis through induction of TIMP1 and decreased activation of MMP2. However, the unique therapeutic potential of tocotrienols is not limited to neuroprotection. Tocotrienols have been shown to have molecular targets including: apoptotic regulators, cytokines, adhesion molecules, enzymes, kinases, receptors, transcription factors, and growth factors. In spite of this large and unique therapeutic potential, scientific literature on tocotrienols only accounts for approximately 1% of vitamin E research. Given the potential of tocotrienols and relatively scant literature, further investigation is warranted.

Keywords: Arteriogenesis, MMP2, Neuroprotection, TIMP1, Tocotrienols, Vitamin E

Discovery of Vitamin E in 1922 is credited to Herbert Evans and Katherine Scott Bishop when they found that “antisterility factor x” was essential for reproduction in rats¹. The pair showed that rats fed a purified diet of casein (18%), cornstarch (54%), lard (15%), butterfat (9%), salts (4%), adequate vitamin A (as cod liver oil), vitamin B (as yeast), and vitamin C (as orange juice) lost their ability to reproduce². Two years later Barnett Sure confirmed Evans and Bishop’s observations after performing similar experiments; he called the substance “vitamin E” because vitamins A, B, C, and D were then already known to the scientific community². In 1936 Evans first published the chemical formula of Vitamin E in the *Journal of Biological Chemistry*¹.

Thirty seven years following the discovery of vitamin E, Green, McHale, Marcinkiewicz, Mamalis, and Watt (1959) discovered 5,7,8-trimethyltocotrienol (the unsaturated derivative of α -tocopherol) in rice³. In 1961, Bunyan *et al.*³ coined the term “tocotrienol” for 2-methyl-2-(4',8',12'-trimethyltrideca-3,7,11-trienyl)-6-chromanol, “the unsaturated derivative of tocol”. Though the discovery of tocotrienol was a significant milestone, it was also the first time the biologic significance of this form of vitamin E was discounted. Bunyan *et al.*³ stated: “the results of tests involving oral dosing

give roughly similar results, suggesting that the general order of potency is trimethyl- > dimethyl- > monomethyl-tocol, with the unsaturated derivatives of α - and β -tocopherol less active than the corresponding saturated compounds”.

Today natural vitamin E is composed of 8 members, divided into 2 classes; tocopherols (TCP) and tocotrienols (TCT). Tocopherols are characterized by a saturated phytyl side chain with 3 chiral carbons, whereas tocotrienols possess a farnesyl side chain with double bonds at carbons 3, 7, and 11. Isomers of tocopherol and tocotrienol are differentiated according to the position and degree of methylation on the chromanol head (α , β , γ , and δ)⁴⁻⁷. Tocopherols represent the primary form of vitamin E in green leafy vegetables, while tocotrienols are found in highest concentration in seeds of monocots that include wheat, rice, barley, and palm⁸.

Molecular targets and biological significance

Tocopherols and tocotrienols share the same vitamin E family, however emergent literature has identified unique biological functions of tocotrienols that are not shared by tocopherols⁶. In particular, α -, and γ -tocotrienol have emerged as vitamin E molecules with neuroprotective and anticancer properties not exhibited by α -tocopherol^{5,9}. Tocotrienols have been shown to induce apoptosis and inhibit proliferation in many tissues including breast, colon, liver, lung, stomach, skin, pancreas,

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and prostate¹⁰. In addition, oral administration of δ -tocotrienol significantly accumulates in tumors of mice with pancreatic cancer at a concentration known to inhibit tumor growth¹¹. Importantly, many of the unique therapeutic effects of the tocotrienol isomers occur at a concentration range achievable by dietary supplementation. Nanomolar concentration of α -tocotrienol (α -TCT) in brain tissue of spontaneously hypertensive rats has been achieved by oral supplementation and attenuates ischemic stroke-induced brain damage¹².

All forms of vitamin E have antioxidant potential however, tocotrienols have been shown to have more potent antioxidant potential than tocopherols^{13,14}. Compared to tocopherols, unsaturation of the side chain of tocotrienol confers a different three-dimensional molecular structure leading to changes in the orientation and organization of membrane phospholipids. When compared to α -TCP, α -TCT also possess higher mobility through membranes and therefore has greater lipid-phase antioxidant potency¹⁵.

The effects of tocotrienols are not alone due to their antioxidant potential. Induction of apoptosis in tumor cells occurs through both the extrinsic and intrinsic pathways. In particular, tocotrienols have been shown to induce death receptors¹⁶ and activation of caspase-8 (Refs 10 and 17). In addition, tocotrienol mediated activation of the intrinsic pathway involves upregulation of Bax, cleavage of Bid, release of cytochrome C, and activation of caspase-9 (Ref. 10). Other anti-tumor activity involves apoptosis through DNA fragmentation^{18,19}, upregulation of P53 (Ref. 20) and suppression of VEGF expression^{21,22} and signaling²³⁻²⁵. A recent review¹⁰ summarizes the many molecular targets tocotrienol effects including kinases, receptors, apoptotic regulators, transcription factors, growth factors, enzymes, cytokines, and adhesion molecules, many of which are unique to tocotrienol form of vitamin E. Tocotrienol targets include 12-lipoxygenase (LOX), c-Src kinase, and HMG-coA reductase^{1,10}. Micromolar amounts of tocotrienols have been shown to suppress HMG-coA reductase, the rate limiting step in cholesterol synthesis^{26,27}. In addition, α -TCT has been shown to be a potent inhibitor of 12-lipoxygenase in neural cells subject to glutamate-mediated neurotoxicity²⁸. α -TCT has also been shown to inhibit glutamate activation of phospholipase 2 (PLA₂), thereby affording protection to glutamate induced neuronal death²⁹.

Safety of tocotrienols

Palm oil, a major source of TCT, has a long history of safe consumption in humans¹. Moreover, trials involving oral supplementation of TCT in humans have not demonstrated any serious adverse side effects^{30,31}. Additionally, TCT is a nutrient that is certified by the US FDA to be Generally Recognized As Safe (GRAS;GRN307) and not a drug with potential side effects³². However, the efficacy and safety of the tocopherol form of vitamin E has recently been called into question. Of note, α -TCP has been shown not to be effective in the reduction of overall major cardiovascular events or cancer³³, and has been shown to increase all cause mortality³⁴. Unfortunately these clinical trials and meta-analyses done with α -TCP have been used to extrapolate safety and efficacy to vitamin E in general^{33,35}. Therefore, more studies of tocotrienols are warranted to determine their efficacy and safety.

Vitamin E transport

Compared to tocopherols, availability of tocotrienols represent a substantially reduced proportion of vitamin E available in plants^{1,10}. Also, commonly used vegetable oils including corn, olive, peanut, sesame, soybean, and sunflower contain exclusively tocopherols¹. However, rice bran, palm, and annatto oils are being recognized as rich sources of tocotrienols¹⁰. Palm oil, which naturally contains 30% tocopherols and 70% tocotrienols as its vitamin E content, is slowly gaining acceptance as a natural hydrogenated fat substitute in the United States¹.

Efforts to understand the transport of dietary vitamin E in the past 2 decades have focused on α -TCP transport³⁶⁻³⁹. This led to the discovery of α -tocopherol transfer protein (TTP), which has been identified to mediate α -TCP secretion into plasma³⁷. α -TCP selectively binds to TTP and the affinity of TTP to bind α -TCT is an order of magnitude lower than that of α -TCP. This observation raised concerns about the bioavailability of orally taken TCT in tissues⁴⁰⁻⁴². Such concerns were strengthened by the report that TCT supplemented in laboratory chow does not reach the brain⁴³. However, more recent data demonstrates that TCT taken orally does achieve micromolar concentrations in human blood^{44,45}, and is delivered to vital organs such as brain in sufficient quantities to prevent stroke-induced lesions¹². Although circulatory tocotrienols are lower in

concentration than tocopherols by about 10-fold, plasma α -TCT concentration reaching the micromolar range is an order of magnitude in excess of the concentration required for complete neuroprotection^{28,46}. Moreover, it has been demonstrated that oral supplementation of tocotrienol to mice and rats is effectively delivered to vital organs including the brain, liver, heart, skin, lungs, adipose tissue, and whole blood. In fact, experimental studies using TTP^{-/-}(knockout) mice clearly demonstrate that orally supplemented tocotrienols are transported to vital organs even in the absence of TTP⁸. It has also been well documented that tocopherol deficiency in TTP^{-/-}mice leads to a loss of reproduction^{47,48}. However, it has been clearly demonstrated that fertility can be restored by supplementation of tocotrienols in TTP^{-/-}mice⁸. These findings strongly demonstrate the existence of TTP-independent mechanisms of transport for tocotrienol taken orally and warrant further investigation.

Ataxia with vitamin E deficiency (AVED) is an autosomal recessive disease in humans caused by mutations in the TTP gene on chromosome 8q13 (Refs 49 and 50). This disease is characterized by low plasma α -TCP concentration and is associated with pathological conditions that include Friedreich-like ataxia and retinitis pigmentosa subsequent to ataxia. Neurological symptoms include ataxia, dysarthria, hyporeflexia, and decreased proprioceptive and vibratory sensations. TTP deficiency in humans specifically affects the central axons of dorsal root ganglion cells and retina, with minor involvement of the peripheral sensory nerves, optic nerves, and pyramidal tracts^{49,51}. High dose α -TCP supplementation, aimed at delivering TCPs independent of TTP, improves neurological outcome in AVED patients, however, recovery is slow and incomplete⁵². TCT supplementation which acts independent of TTP⁸ may be beneficial in this special human population with TTP deficiency. Further studies in this area are needed.

Neuroprotection

Recent studies on the neuroprotective properties of tocotrienols have established the significance of α -TCT as the most potent neuroprotective form of vitamin E³². It has been reported that α -TCT and not α -TCP prevents stroke-associated neurodegeneration^{12,46}. Brain tissue is highly sensitive to oxidative stress, and in particular neurons are

vulnerable to oxidative damage due to lower levels of endogenous antioxidant glutathione as compared to resident glial cells^{1,53}. The generation of free radicals in injured brain tissue leads to lipid peroxidation of polyunsaturated fatty acids (PUFAs) which have been proven to modulate the cell death cascade. The brain is highly enriched with n-6 PUFA arachidonic acid (AA) and n-3 PUFA docosahexaenoic acid (DHA) which comprise 20% of all fatty acids in the mammalian brain¹. Recent evidence supports the role of α -TCT as a protective agent in the AA cascade of neurodegeneration with an ability to target both enzymatic and non-enzymatic mechanisms of injury¹ (Fig. 1). Protection of neuronal cells by α -TCT is mediated by inhibition of inducible c-Src, inhibition of activation of PLA₂, inhibition of 12-lipoxygenase^{1,12,54}, upregulation of multidrug resistance protein 1 (MRP1)³², induction of tissue inhibitor of metalloprotease 1 (TIMP1), attenuation of matrix metalloprotease 2 (MMP2)⁵⁵, and through the antioxidant capacity of tocotrienols¹.

Antioxidative neuroprotection—Reactions of free radical species with double bonds of PUFAs produce alkyl radicals, which react with molecular oxygen to form a peroxy radical. The peroxy radical propagates a chain reaction of lipid oxidation¹. Lipid peroxides subsequently degrade and give rise to unsaturated aldehydes, these aldehydes covalently bind to proteins through reaction with thiol groups and alter their function. Vitamin E, the major chain-breaking antioxidant is the first line of defense against peroxidation of lipid membranes¹. Compared to tocopherols, tocotrienols are thought to have superior antioxidant capacity¹⁴. Unsaturation of phytol tail of tocotrienols not only affords a different

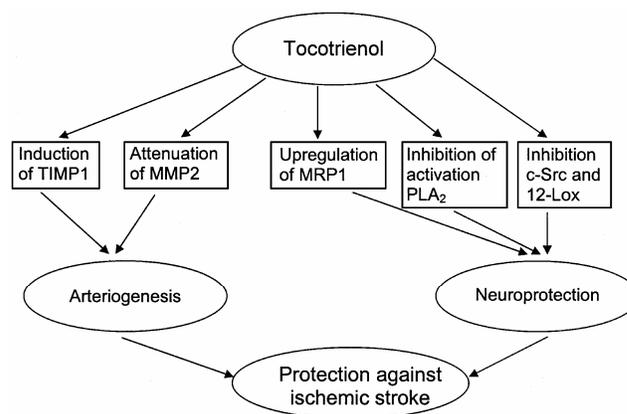


Fig. 1—Effects of tocotrienol on molecular targets leading to protection against ischemic stroke.

3-dimensional structure than tocopherols, but also superior penetration into tissues with saturated fatty layers, like brain. These properties of TCT may contribute to easier access of ascorbate to reduce an oxidized α -TCT radical therefore, enhancing antioxidant regeneration of α -TCT more effectively than α -TCP in brain^{1,56}.

Phospholipase A₂—The phospholipase A₂ (PLA₂) family of isozymes is responsible for enzymatic hydrolysis of sn-2 ester bond of glycerophospholipids resulting in a free fatty acid (i.e., arachidonic acid; AA) and lysophospholipid (i.e., lysophosphatidylcholine). Currently, only secreted PLA₂ (sPLA₂) and cytosolic PLA₂ (cPLA₂) have well defined roles in pathologic arachidonic acid metabolism¹. Under conditions of ischemic stroke, sPLA₂ mRNA and protein expression are significantly upregulated^{57,58}. Additionally, cPLA₂s are the only PLA₂ that demonstrate a preference for AA at the sn-2 position of phospholipids^{1,59}. In the pathologic setting, free AA accumulates and undergoes uncontrolled oxidative metabolism by both enzymatic and non-enzymatic processes. Arachidonic acid metabolism includes formation of harmful prostaglandins, leukotrienes, thromboxanes, isoprostanes, and non-enzymatic lipid peroxidation products¹. It has been shown that α -TCT attenuates cPLA₂ activity under glutamate mediated toxicity in neural cells²⁹. Glutamate activates cPLA₂ in neurons in a calcium-dependent manner, leading to hydrolysis of AA from phospholipids^{1,29}. Both phosphorylation and translocation of cPLA₂ are inhibited with nanomolar concentration of α -TCT, levels achievable by oral supplementation^{8,60}.

12-LOX and c-Src—The enzymatic generation of oxygenated derivatives of arachidonic acid (AA) is mediated through cyclooxygenase, epoxygenase, and LOX enzymes. In particular the LOX pathway has been identified as a key mediator of neurodegeneration and cell death¹. Three forms of LOX are present in brain tissue, 5-, 12- and 15-LOX, of which 12-LOX is the most abundant. Of interest, 12-LOX deficient mice are resistant to ischemic stroke injury¹² and α -TCT has been shown to be a potent 12-LOX inhibitor in neuronal cells subjected to glutamate-induced neurotoxicity²⁸. α -TCT directly interacts with 12-LOX to suppress AA metabolism, and *in silico* studies suggest the presence of an α -TCT binding site on 12-LOX close to the active site with the potential to block AA access¹. Deficiency or inhibition of c-Src in

mice protects brain tissue from stroke-induced neurodegeneration as well^{1,61}. Relevance of c-Src in neurodegeneration comes from rapid activation of c-Src in glutamate challenge leading to phosphorylation of 12-LOX. Glutamate-induced c-Src activity is blocked by nanomolar amounts of α -TCT⁴⁶. The effects of α -TCT on c-Src and 12-LOX suggest that α -TCT is a potent inhibitor of 12-LOX mediated AA metabolism and neurodegeneration.

Multidrug resistance protein 1 (MRP1)—Recently the role of intracellular GSSG mediated cell death has been reported⁶². Glutathione (GSH) is the major endogenous antioxidant produced by cells and under normal physiologic states the reduced form of glutathione predominates (99%) over the oxidized form (GSSG, 1%)³². During conditions of great oxidative stress GSH is rapidly oxidized to GSSG, this reaction while reversible requires reducing equivalents like vitamin C and E which may be depleted in a pro-oxidant state. Excessive GSSG is toxic to cells and therefore, energy is expended to pump it out of the cell. Ineffective clearance of GSSG triggers neuronal cell death³². Multidrug resistance-associated protein 1 (MRP1) plays a significant role in clearing GSSG and thus, preventing cell death⁶³. Previously, it has been reported that α -TCT is protective against GSSG-induced neuronal cell death^{32,54,62,64}. A recent study demonstrates that the ability of α -TCT to protect against glutamate challenge is compromised following MRP1 knockdown in primary cortical neurons. Moreover, α -TCT has been shown to upregulate MRP1 in the infarct hemisphere of mice that underwent middle cerebral artery occlusion (MCA) to induce ischemic stroke³². Of great significance, α -TCT significantly reduces hemispherical infarct volume in mice subject to MCA occlusion³².

Tissue inhibitor of metalloprotease 1 (TIMP1) and Multidrug resistance-associated protein 1 (MMP2)—Cerebral ischemia causes failure of ATP-dependent ion transporters resulting in rapid accumulation of intracellular sodium and an influx of water to maintain osmotic equilibrium. This cytotoxic edema is characterized by swelling in the first 24 h of stroke onset⁵⁵. Diffusion tensor imaging (DTI) has shown that TCT supplemented canines subject to middle cerebral artery (MCA) occlusion have significantly reduced cytotoxic edema at 1 h following acute ischemic stroke compared to

canines receiving vitamin E deficient corn oil (placebo)⁵⁵. Moreover, TCT supplementation significantly attenuated ischemic stroke-induced lesion volume and prevented loss of white matter fiber tract connectivity⁵⁵. Post-hoc analysis of cerebral angiograms during MCA occlusion showed that TCT supplemented canines had improved cerebrovascular collateral circulation to ischemic MCA territory⁵⁵. In addition, TCT supplementation induced arteriogenic TIMP1 and subsequently attenuated the activity of MMP2⁵⁵. The observation that TCT induces TIMP1 expression in blood vessels suggests that long-term orally supplemented TCT may prime the cerebral vasculature enabling adaptive arteriogenesis in response to focal cerebral ischemia⁵⁵.

Failure of α -TOC in clinical trials testing a wide range of diseases⁶⁵ has increased scientific awareness and interest in tocotrienol research. However, tocotrienols continually suffer a fate bestowed upon them by the much better known tocopherols, as demonstrated by a meta-analysis including 9 trials and 118,765 subjects testing the effect of vitamin E on stroke subtypes and incident stroke⁶⁶. The authors demonstrated that vitamin E had no effect on the risk for total stroke. The subjects did, however, have a reduction in the risk of ischemic stroke (relative risk 0.90), but unfortunately demonstrated an increased risk for hemorrhagic stroke (relative risk 1.22). In terms of absolute risk, for every 1250 individuals taking vitamin E one additional hemorrhagic stroke occurred. For every 476 patients taking vitamin E one ischemic stroke was prevented⁶⁶. However, it should be noted that the clinical trials involved in the meta-analysis only studied the effect of the tocopherol form of vitamin E. The authors concluded that: "indiscriminate widespread use of vitamin E should be cautioned against" due to the generally more severe outcome of hemorrhagic stroke⁶⁶. The authors do comment on weaknesses of the study, of note they included randomized control trials irrespective of blinding and morbidity status of participants. In addition the authors do give consideration to different effects of synthetic and natural "vitamin E" however, when making claims as for all forms of vitamin E, the authors do not give consideration to tocotrienols. Given the growing body of literature in support of unique biological functions across vitamin E family members, title claims of research should be limited to the specific isoform of study.

Conclusion

Emergent literature addressing the tocotrienol form of vitamin E has clearly identified unique biological functions independent of antioxidant activity that are not shared by the well characterized α -TCP isoform. As meta-analyses of clinical trials testing the effects of tocopherols in disease are drawing major conclusions relevant to public health, ongoing research in tocotrienol address a blind spot in vitamin E research. Therefore, the efficacy and safety of tocotrienol should be determined by the study of these vitamins and not through extrapolation of studies conducted on other forms of vitamin E. As discussed here, tocotrienols possess many unique properties including the neuroprotective qualities of α -TCT with specific molecular targets (cPLA₂, 12-LOX, c-Src, TIMP1, MMP2, and MRP1) and mechanisms of action. Tocotrienols have also demonstrated therapeutic promise in the treatment of cancer and hyperlipidemia. Research on tocotrienol transport already points to a TTP independent mechanism of transport and further research in this area may lead to additional therapeutic potential for people with AVED. In conclusion the potential of tocotrienols as a therapeutic agent is a significant one and warrants further investigation.

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