Symposium: Molecular Mechanisms of Protective Effects of Vitamin E in Atherosclerosis

Molecular Aspects of α-Tocotrienol Antioxidant Action and Cell Signalling

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ABSTRACT Vitamin E, the most important lipid-soluble antioxidant, was discovered at the University of California at Berkeley in 1922 in the laboratory of Herbert M. Evans (Science 1922, 55: 650). At least eight vitamin E isoforms with biological activity have been isolated from plant sources. Since its discovery, mainly antioxidant and recently also cell signaling aspects of tocopherols and tocotrienols have been studied. Tocopherols and tocotrienols are part of an interlinking set of antioxidant cycles, which has been termed the antioxidant network. Although the antioxidant activity of tocotrienols is higher than that of tocopherols, tocotrienols have a lower bioavailability after oral ingestion. Tocotrienols penetrate rapidly through skin and efficiently combat oxidative stress induced by UV or ozone. Tocotrienols have beneficial effects in cardiovascular diseases both by inhibiting LDL oxidation and by down-regulating 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG CoA) reductase, a key enzyme of the mevalonate pathway. Important novel antiproliferative and neuroprotective effects of tocotrienols, which may be independent of their antioxidant activity, have also been described. J. Nutr. 131: 369S–373S, 2001.

KEY WORDS: • tocotrienols • tocopherols • antioxidants • cell signaling

Vitamin E isoforms and their natural sources. Vitamin E occurs in nature in at least eight different isoforms: α-, β-, γ- and δ-tocopherols and α-, β-, γ- and δ-tocotrienols. Tocotrienols differ from the corresponding tocopherols only in their aliphatic tail. Tocopherols have a phytol side chain attached to their chromanol nucleus, whereas the tail of tocotrienols is unsaturated and forms an isoprenoid chain (Fig. 1). The various isoforms of tocotrienols differ in their methyl substituents on the chromanol nucleus. The α-form contains 3 methyl groups, whereas the β- and γ- have two and the δ-form only one methyl group. Each of these forms of vitamin E has a reportedly different biopotency (Azzi and Stocker 2000, Brigelius-Flohe and Traber 1999, Traber and Packer 1995). Humans absorb all forms of vitamin E, but the body maintains only α-tocopherol; this is the basis of the new recommended dietary allowances of vitamin E, which define the human requirement only for α-tocopherol (International Institute of Medicine 2000).

Lipid-rich plant products and vegetable oils are the main natural sources of vitamin E (see Table 1). Tocotrienols are found in high concentrations in palm oil and rice bran (Theauralt et al. 1999). Other natural sources include coconut oil, cocoa butter, soybeans, barley and wheat germ. Moreover, tocotrienols were also detected in meat and eggs. Sunflower, peanut, walnut, sesame and olive oils, however contain only tocopherols (Heinonen and Pironen 1991).

Antioxidant activity of tocotrienols. Vitamin E is incorporated into cellular membranes in which it effectively inhibits the peroxidation of lipids. Both tocopherols and tocotrienols scavenge the chain-propagating peroxyl radical. When comparing the effectiveness of different vitamin E homologues, at least two factors must be considered, i.e., the substituents on the chromanol nucleus and the properties of the tail chain. In homogeneous solutions, the reaction rate constant depends mainly on the number of methyl groups on the nucleus. In membranes, the mobility of the molecule also becomes important, and this depends on the structure of the hydrophobic side chain. Although no difference in radical-scavenging activity between α-tocopherol and α-tocotrienol was found in hexane, the activity of α-tocotrienol in scavenging peroxyl radicals is 1.5-fold higher in liposomes compared with α-tocopherol (Serbinova et al. 1991). In rat liver microsomes, the efficacy of α-tocotrienol to protect against Fe(II) + NADPH-induced lipid peroxidation was 40 times higher than that of α-tocopherol. α-Tocotrienol also was 6.5 times more effective in the protection of cytochrome P-450 against oxidative damage. Several reasons have been suggested for the increased antioxidant activity of α-tocotrienol vs. α-tocopherol, focusing on the differences in the tail structure. The chromanolxyl radical of α-tocotrienol (α-tocotrienoxyl) has been found to be recycled in membranes and lipoproteins more quickly than the corresponding α-tocopheroxyl radical (Serbi-
noma et al. 1991). Nuclear magnetic resonance studies have indicated that \( \alpha \)-tocotrienol is located closer to the membrane surface, which may facilitate recycling. Furthermore, \( \alpha \)-tocotrienol has a stronger disordering effect on membranes than \( \alpha \)-tocopherol and is distributed more uniformly within the membrane. These properties likely enhance the interaction of chromanols with lipid radicals (Serbinova et al. 1991, Suzuki et al. 1993). As summarized in Table 2, there is substantial evidence that tocotrienols may be more efficient radical scavengers in biomembranes that the corresponding tocopherols.

Vitamin E does not work in isolation from other antioxidants; rather it is part of an interlinking set of redox antioxidant cycles (Constantinescu et al. 1993), which has been termed the "antioxidant network" (Fig. 2). It is hypothesized that vitamin E acts catalytically, i.e., it is efficiently reduced from its free radical (chromanoxyl) form, which arises after quenching lipid radicals, to return back to its reduced native state. This catalysis occurs through the interactions between water- and lipid-soluble substances by both nonenzymatic and enzymatic mechanisms that regenerate vitamin E from its tocotrienoxyl or tocopheroxyl radical back to tocotrienol and tocopherol, respectively. Vitamin C can regenerate vitamin E directly, and thiol antioxidants, such as glutathione and lipoic acid, can regenerate vitamin E indirectly via vitamin C. Under conditions in which these systems act synergistically to keep the steady-state concentration of vitamin E radicals low, the loss or consumption of vitamin E is prevented. Such recycling effects can be seen in human LDL (Kagan et al. 1992) and in membranes enriched with tocopherols or tocotrienols or by feeding animals vitamin E–enriched diets (Suarna et al. 1993, Verlangieri and Bush 1992).

Absorption and distribution in tissues. The antioxidant efficacy of tocotrienols in membranes is higher than that of tocopherols, although their uptake and distribution after oral ingestion are less than that of \( \alpha \)-tocopherol. In hamsters fed a mixture of vitamin E isoforms also containing tocotrienols, \( \alpha \)-tocopherol was absorbed preferentially. However, tocotrienols could still be detected in the postprandial plasma of humans, and tocotrienols were found in all classes of lipoproteins (Hayes et al. 1993). The liver contains a transfer protein that preferentially enriches VLDL with \( \alpha \)-tocopherol (Arita et al. 1995). Therefore, \( \alpha \)-tocopherol is secreted preferentially by the liver in a manner that discriminates between tocopherols and tocotrienols. Interestingly, the \( \alpha \)-tocopherol transfer protein (\( \alpha \)-TTP) was identified as a product of the causative gene for familial isolated vitamin E deficiency (Ouahchi et al. 1995). The mRNA of \( \alpha \)-TTP was recently detected at low levels in other tissues including brain, spleen, lung and kidney (Hosomi et al. 1998). The presence of a transfer protein that preferentially selects \( \alpha \)-tocopherol seems to explain why all other forms of vitamin E have a lower biological activity in the gestation-resorption assay compared with \( \alpha \)-tocopherol. Even though tocotrienols have a higher radical-scavenging activity than tocopherols, they are less bioavailable after oral ingestion. It can be hypothesized that if similar tissue levels could be achieved, tocotrienols would be more effective antioxidants than tocopherols. There is some evidence supporting this hypothesis. When supplementation was carried out in a way that allowed comparable tissue concentrations of \( \alpha \)-tocopherol and \( \alpha \)-tocotrienol to be reached in rat microsomes and mitochondria, tocotrienol-supplemented heart tissues were more resistant to lipid peroxidation in vitro than the tocopherol-supplemented counterparts (Serbinova and Packer 1994).

However, it is important to note that tocotrienols belong to a family of plant phenolic compounds, which have a brief and transient nature with respect to their metabolism, i.e., compared with \( \alpha \)-tocopherol, they are inferior with regard to tissue retention and half-life.

The distribution of vitamin E isoforms varies from tissue to tissue. In mice fed a diet not specifically enriched with tocotrienols, up to 15% of total vitamin E was composed of tocotrienols; the brain contained no detectable \( \alpha \)-tocotrienol levels; in other tissues, 99% of the vitamin E was present as \( \alpha \)- or \( \gamma \)-tocopherol (Podda et al. 1996). Similarly, in hamsters, tocotrienols were detected in all tissues except the brain (Hayes et al. 1993). These results indicate that tissues may possess the ability to regulate the vitamin E composition individually. Tocotrienols penetrate rapidly through skin, and its topical application is an efficient means with which to enrich skin with vitamin E (Traber et al. 1998). If skin is exposed to oxidative stress produced by UV or ozone after the

### TABLE 1

<table>
<thead>
<tr>
<th>Source</th>
<th>Tocotrienols</th>
<th>Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm</td>
<td>14.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Rice bran</td>
<td>23.6</td>
<td>NA</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>2.6</td>
<td>18.1</td>
</tr>
<tr>
<td>Coconut</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Soybean</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Olive</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Sources: Sheppard et al. (1993), Ong (1993).
2 NA, not analyzed.

### TABLE 2

Factors determining higher antioxidant activity of \( \alpha \)-tocotrienol compared with \( \alpha \)-tocopherol

<table>
<thead>
<tr>
<th>Greater antioxidant activity of ( \alpha )-tocotrienol results from the following:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. More uniform distribution in membrane bilayer</td>
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<tr>
<td>2. Stronger disordering of membrane lipids</td>
</tr>
<tr>
<td>3. More effective collision with radicals</td>
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<tr>
<td>4. Greater recycling activity of chromanoxyl radical</td>
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<tr>
<td>5. Recycling activity correlates with inhibition of lipid peroxidation</td>
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</tbody>
</table>

2 Abbreviations: \( \alpha \)-TTP, \( \alpha \)-tocopherol transfer protein; GSH, glutathione; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; PKC, protein kinase C; ROS, reactive oxygen species; TRF, tocotrienol-rich fraction of palm oil.
has been reported recently that humans do not respond uniformly to the cholesterol-lowering action of tocotrienols, particularly when cholesterol and alcohol intakes are not controlled (Qureshi et al. 1997).

**Evidence for molecular and cell biological aspects of tocopherol and tocotrienol on signal transduction**

**Anticarcinogenic properties.** Tocotrienols belong to a phytochemical class of isoprenoid molecules. These compounds share a common precursor, mevalonoid acid. Tocotrienols are mixed isoprenoids, meaning that only a part, the lipophilic chain, is derived via the isoprenoid pathway. Isoprenoids have been shown to exhibit anticarcinogenic properties. When different vitamin E isoforms were analyzed, it could be demonstrated that α-tocopherol and α-tocotrienol inhibited tumor promotion in Raji-cells most effectively (Goh et al. 1994). Tocotrienols from TRF inhibited the proliferation of human breast cancer cell lines (Guthrie 1997, Nesaretnam et al. 1995). The inhibition was found to be independent of the estrogen receptor status of the cell lines (Nesaretnam et al. 1998). Isoprenoids, including tocotrienols, also suppressed the growth of murine B16 melanomas in vitro and in vivo (He et al. 1997). Interestingly, correlations between the late-stage tumor-suppressive potency of diverse isoprenoids and their effect of HMG CoA reductase activity approached unity. It is hypothesized that vitamin E might exert antiproliferative properties by interfering with signal transduction events involving protein kinase C (PKC). It is has been shown that α-tocopherol inhibits the proliferation of smooth muscle cells by inhibition of PKC (Tasinato et al. 1995). This effect was specific for α-tocopherol as opposed to the isoform β-tocopherol (Azzi et al. 1993). There is no information, however, on the potency of α-tocotrienol on PKC activity, which shares the structure of the chromanol nucleus with α-tocopherol. Recently, it has been reported that isoprenoids, including tocotrienols, induce cell-cycle arrest in the G1 phase and apoptosis in human and murine tumor cells (Yu et al. 1999). Because these effects can be observed with different isoprenoids, which are not antioxidants, it is possible that the anticarcinogenic effects of tocotrienols are not necessarily related to their antioxidant properties.

**Inhibition of cholesterol synthesis.** A protective effect in cardiovascular diseases has been attributed to vitamin E (Meydani 1995). In this scenario, tocotrienols may exert protective effects exceeding those of tocopherols. Cell culture studies indicate clearly that tocotrienols influence cholesterol synthesis by directly regulating the expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), principally through a posttranscriptional process involving accelerated degradation of the reductase protein (Parker et al. 1993). In pigs with inherited hyperlipidemia, dietary tocotrienols from a tocotrienol-rich fraction of palm oil (TRF) reduced the concentrations of plasma cholesterol and apolipoprotein B, thromboxane B2, and platelet factor 4, indicating a protective effect on endothelium and platelet aggregation (Qureshi et al. 1991a). When rats were fed an atherogenic diet, both α-tocotrienol and α-tocopherol significantly lowered plasma lipid concentrations (Watkins et al. 1993). Moreover, supplementation with TRF reduced plasma cholesterol levels in a human pilot study lasting 8 wk (Qureshi et al. 1991b). In a trial of 4 wk, these results were confirmed, and a carry-over effect after the end of supplementation was reported (Qureshi et al. 1995). Interestingly, dietary α-tocopherol attenuated the cholesterol-lowering effect of α-tocotrienol in both humans and chickens (Qureshi et al. 1995 and 1996). In a double-blind, placebo-controlled trial, no effect of a vitamin E supplement rich in tocotrienols (140 mg/d for 6 wk) on serum lipids, lipoproteins or platelet function in men with mildly elevated serum lipid concentrations was found (Mensink et al. 1999). Furthermore, α-tocotrienyl acetate, which is hydrolyzed, absorbed and detectable in human plasma, did not lower cholesterol in hypercholesterolemic subjects but was potent in decreasing LDL oxidizability (O’Byrne et al. 2000). Some of the conflicting results in the literature regarding the cholesterol-lowering effects of tocotrienols might be related to differences in plasma tocotrienol levels. In vitro studies with HepG2 cells suggest that tocotrienols are effective at levels of 10 μmol/L (Parker et al. 1993), which may not have been reached in the human trials summarized above. Moreover, it has been reported recently that humans do not respond uni-
Neuroprotection and src activity. Elevated levels of glutamate have been implicated in a wide range of neurological diseases, including epilepsy, cerebral ischemia, Huntington’s disease and Parkinson’s disease. Receptor-mediated glutamate excitotoxicity is believed to be a major mechanism of damage in these pathologies, and induction of oxidative stress by glutamate has been demonstrated to be the primary cytotoxic mechanisms in cell lines such as C6 glial cells (Han et al. 1997), PC-12 neuronal cells (Pereira and Oliveira 1997), and immature cortical neuron cells (Murphy et al. 1990) and oligodendroglia cells (Oka et al. 1993). It has been demonstrated that high glutamate levels block cystine uptake via amino acid transporter Xc–, resulting in a significant depletion of cellular glutathione (GSH). A GSH-depleted state impairs cellular antioxidant defenses, followed by an increased vulnerability of the cell to reactive oxygen species (ROS). The mitochondrial electron transport chain has been shown to be a source of ROS production during glutamate-induced apoptosis (Tan et al. 1998). Recently, vitamin E isoforms were tested in a model of neuronal cell death in which HT4 neuronal cells were challenged with glutamate (Sen et al. 2000). Tocotrienols counteracted glutamate-induced cell death at much lower concentrations than tocopherols. Moreover, tocotrienols effectively inhibited the activation of pp60c-src kinase, a kinase that is centrally involved in glutamate-induced cell death. It is hypothesized that these protective effects of tocotrienols are probably independent of their antioxidant activity because tocopherols were effective only at multifold higher concentrations (Sen et al. 2000). The activity of src kinase has also been shown in the progression of breast cancer (Muthuswamy and Muller 1995). Elevated levels of src kinase have also been found in human skin tumors (Barnekow et al. 1987). Because of the key involvement of src kinase in neurodegenerative diseases and oncogenesis, inhibition of these kinases would seem to be a likely basis for developing a strategy to create neuroprotective and anticancer drugs.

Tocotrienols make up a considerable portion of total vitamin E in many food sources. In vitro, they have been shown to exhibit enhanced antioxidant properties compared with tocopherols. In addition, they have been shown to have cholesterol-lowering, anticarcinogenic and neuroprotective properties, which may not be related to their antioxidant function. After oral ingestion, however, they are not recognized by the gastrointestinal tract and intestinal absorption is limited. Therefore, tocotrienols are not efficiently absorbed from the gut and their bioavailability is low. A promising approach to utilize tocotrienols is to administer tocotrienol-rich vitamin E concentrate rich in tocotrienols. In human subjects, 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. 21: 213–219.


