Molecular Aspects of \(\alpha\)-Tocotrienol Antioxidant Action and Cell Signalling\(^1\)

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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-hydroxy-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: \(\alpha\)-tocotrienols \(\alpha\)-tocopherols antioxidants cell signaling

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TABLE 1

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</table>

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

TABLE 2

Factors determining higher antioxidant activity of α-tocotrienol compared with α-tocopherol

Greater antioxidant activity of α-tocotrienol results from the following:
- More uniform distribution in membrane bilayer
- Stronger disordering of membrane lipids
- More effective collision with radicals
- Greater recycling activity of chromanol radical
- Recycling activity correlates with inhibition of lipid peroxidation

 tocopherols, although their uptake and distribution after oral ingestion are less than that of α-tocopherol. In hamsters fed a mixture of vitamin E isoforms also containing tocotrienols, α-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 α-tocopherol (Arita et al. 1995). Therefore, α-tocopherol is secreted preferentially by the liver in a manner that discriminates between tocophersols and tocotrienols. Interestingly, the α-tocopherol transfer protein (α-TTP) was identified as a product of the causative gene for familial isolated vitamin E deficiency (Ouahchi et al. 1995). The mRNA of α-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 α-tocopherol seems to explain why all other forms of vitamin E have a lower biological activity in the gestation-resorption assay compared with α-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 α-tocopherol and α-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 α-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 α-tocotrienol levels; in other tissues, 99% of the vitamin E was present as α- or γ-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
topical application of vitamin E, the increased antioxidant content is sufficient to combat oxidative stress (Thiele et al. 1997, Weber et al. 1997).

**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 postranscriptional 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. 1999a). When rats were fed an atherogenic diet, both \( \alpha \)-tocotrienol and \( \alpha \)-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. 1999b). 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. 1999). Interestingly, dietary \( \alpha \)-tocopherol attenuated the cholesterol-lowering effect of \( \alpha \)-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, \( \alpha \)-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’Brien 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 \( \mu \text{mol/L} \) (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 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 the phytochemical class of isoprenoid molecules. These compounds share a common precursor, mevalonic 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 \( \alpha \)-tocopherol and \( \alpha \)-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 \( \alpha \)-tocopherol inhibits the proliferation of smooth muscle cells by inhibition of PKC (Tasinato et al. 1995). This effect was specific for \( \alpha \)-tocopherol as opposed to the isofom \( \beta \)-tocopherol (Azzi et al. 1993). There is no information, however, on the potency of \( \alpha \)-tocotrienol on PKC activity, which shares the structure of the chromanol nucleus with \( \alpha \)-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.
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), immature cortical neuron 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 tocophersols. Moreover, tocotrienols effectively inhibited the activation of pp60 c-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 tocophersols 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 tocophersols. 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 α-TTP and thus only have a short half-life, which accounts for their low bioavailability. A promising approach to utilize tocotrienols may be the topical application onto the skin. In this scenario, uptake and distribution within the skin do not depend on transfer proteins, thereby allowing active concentrations to be reached in skin after topical supplementation.

LITERATURE CITED

Johansen, M. (1995) Ataxia with isolated vitamin E deficiency is caused by mutations in the α-TTP and thus only have a short half-life, which accounts for their low bioavailability. A promising approach to utilize tocotrienols may be the topical application onto the skin. In this scenario, uptake and distribution within the skin do not depend on transfer proteins, thereby allowing active concentrations to be reached in skin after topical supplementation.


