Tocotrienol

The Natural Vitamin E to Defend the Nervous System?

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ABSTRACT: Vitamin E is essential for normal neurological function. It is the major lipid-soluble, chain-breaking antioxidant in the body, protecting the integrity of membranes by inhibiting lipid peroxidation. Mostly on the basis of symptoms of primary vitamin E deficiency, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function. Orally supplemented vitamin E reaches the cerebrospinal fluid and brain. Vitamin E is a generic term for all tocopherols and their derivatives having the biological activity of RRR-α-tocopherol, the naturally occurring stereoisomer compounds with vitamin E activity. In nature, eight substances have been found to have vitamin E activity: α-, β-, γ- and δ-tocopherol; and α-, β-, γ- and δ-tocotrienol. Often, the term vitamin E is synonymously used with α-tocopherol. Tocotrienols, formerly known as ζ, ϵ, or η-tocopherols, are similar to tocopherols except that they have an isoprenoid tail with three unsaturation points instead of a saturated phytyl tail. Although tocopherols are predominantly found in corn, soybean, and olive oils, tocotrienols are particularly rich in palm, rice bran, and barley oils. Tocotrienols possess powerful antioxidant, anticancer, and cholesterol-lowering properties. Recently, we have observed that α-tocotrienol is multi-fold more potent than α-tocopherol in protecting HT4 and primary neuronal cells against toxicity induced by glutamate as well as by a number of other toxins. At nanomolar concentration, tocotrienol, but not tocopherol, completely protected neurons by an antioxidant-independent mechanism. Our current work identifies two major targets of tocotrienol in the neuron: c-Src kinase and 12-lipoxygenase. Dietary supplementation studies have established that tocotrienol, fed orally, does reach the brain. The current findings point towards tocotrienol as a potent neuroprotective form of natural vitamin E.

KEYWORDS: nutrient; glutamate; neurotoxicity; antioxidant; neuroprotection

Vitamin E is essential for normal neurological function.[1,2] The nervous system is vulnerable to the damaging effects of highly reactive free radicals for several reasons. The brain contains high amounts of polyunsaturated (20:4 and 22:6) fatty acids

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that are susceptible to lipid peroxidation, receives a large percentage of oxygen, and is relatively deficient in certain antioxidant enzymes. In addition, specific regions of the brain have high iron concentrations. Thus, antioxidant defenses are critically important to protect the brain and neural tissues from oxidative damage.[3] Indeed, numerous pathophysiological conditions have been associated with increased levels of oxidative stress indices.[4–6]

Neuroprotection by antioxidants has therefore drawn much interest. The majority of the available research on the role of antioxidant nutrients in neurological function and disease has focused on vitamin E. Vitamin E is the major lipid-soluble, chain-breaking antioxidant in the body, protecting the integrity of membranes by inhibiting lipid peroxidation. Mostly on the basis of symptoms of primary vitamin E deficiency, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function.[2] Orally supplemented vitamin E reaches the cerebrospinal fluid and brain.[7] One of the most extensively studied aspects of vitamin E is its antioxidant property. Most of the vitamin E–sensitive neurological disorders are associated with elevated levels of oxidative damage markers. This has led to a popular hypothesis stating that the neuroprotective effects of vitamin E are wholly mediated by its antioxidant property.[8]

THE VITAMIN E FAMILY: TOCOPHEROLS AND TOCOTRIENOLS

Vitamin E is a generic term for all tocopherols and their derivatives having the biological activity of RRR-α-tocopherol, the naturally occurring stereoisomer compounds with vitamin E activity.[9,10] In nature, eight substances have been found to have vitamin E activity: α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol (FIG. 1). Often, the term vitamin E is synonymously used with α-tocopherol. Although *d*-α-tocopherol (RRR-α-tocopherol) has the highest bioavailability and is the standard against which all the others must be compared, it is only one out of eight natural forms of vitamin E. Tocotrienols, formerly known as ζ, ε or η-tocopherols (FIG. 1), are similar to tocopherols except that they have an isoprenoid tail with three unsaturated points instead of a saturated phytyl tail (FIG. 1). Although tocopherols are predominantly found in corn, soybean, and olive oils, tocotrienols are particularly rich in palm, rice bran, and barley oils.[9,10]

Interestingly, tocotrienols possess powerful antioxidant, anticancer, and cholesterol-lowering properties (TABLE 1). Some studies have confirmed that tocotrienol activity as an antioxidant, anticancer, and cholesterol-reducing substance to be stronger than tocopherols. Tocotrienols are thought to have more potent antioxidant properties than α-tocopherol.[43,52] The unsaturated side-chain of tocotrienol allows for more efficient penetration into tissues, such as the brain and liver, that have saturated fatty layers.[36] Experimental research examining the antioxidant, free-radical scavenging effects of tocopherol and tocotrienols revealed that tocotrienols appear superior because of their better distribution in the fatty layers of the cell membrane.[36] Although tocotrienols have shown better beneficial effects than α-tocopherol in a limited number of situations, as indicated in the foregoing text, little is known about the exact mechanism of action. Among the pathophysiological situations cited above, only one mechanism of action that accounts for the hypocholesterolemic property of tocotrienol has been characterized. Micromolar amounts of

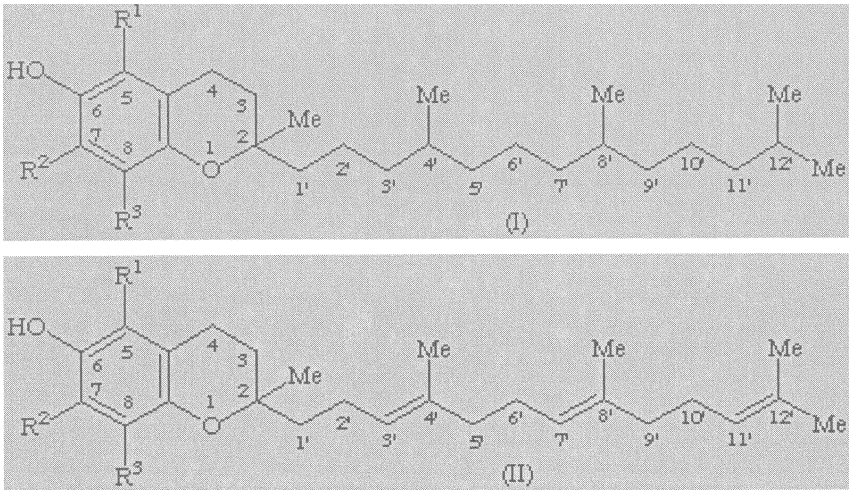

FIGURE 1. Vitamin E: variations and nomenclature. The term tocopherol(s) is a generic descriptor for all monomethyltocols, dimethyltocols, and trimethyltocols. Thus, this term is *not* synonymous with the term vitamin E. Compound I (R1 = R2 = R3 = Me), known as α-tocopherol, is designated α-tocopherol or 5,7,8-trimethyltocol; compound I (R1 = R3 = Me; R2 = H), known as β-tocopherol, is designated, β-tocopherol or 5,8-dimethyltocol; compound I (R1 = H; R2 = R3 = Me), known as γ-tocopherol, is designated γ-tocopherol or 7,8-dimethyltocol; compound I (R1 = R2 = H; R3 = Me), known as δ-tocopherol, is designated δ-tocopherol or 8-methyltocol; compound II (R1 = R2 = R3 = H), 2-methyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-ol, is designated tocotrienol; compound II (R1 = R2 = R3 = Me), formerly known as ζ1 or ζ2-tocopherol, is designated 5,7,8-trimethyltocotrienol or α-tocotrienol. The name tocochromanol-3 has also been used; compound II (R1 = R3 = Me; R2 =), formerly known as ε-tocopherol, is designated 5,8-dimethyltocotrienol or β-tocotrienol; compound II (R1 = H; R2 = R3 = Me), formerly known as η-tocopherol, is designated 7,8-dimethyltocotrienol or γ-tocotrienol. The name plastochromanol-3 has also been used; compound II (R1 = R2 = H; R3 = Me) is designated 8-methyltocotrienol or δ-tocotrienol.[120]

tocotrienol have been shown to suppress the activity of HMG-CoA reductase, the hepatic enzyme responsible for cholesterol synthesis.[33,40]

GLUTAMATE-INDUCED TOXICITY: GENERAL MECHANISMS

Mammalian cells possess an Na^+-independent anionic amino acid transport system, designated as x_c^-, highly specific for cystine and glutamate.[53] This system imports cystine into cells in exchange for glutamate. Cystine taken up by the cell via system x_c^- is rapidly reduced to cysteine, which is incorporated into proteins and glutathione.[54] Because cysteine is a rate-limiting precursor for glutathione synthesis, the intracellular level of glutathione is regulated by the system x_c^- activity.[55] Impaired cellular cystine uptake in human immunodeficiency virus–infected (HIV^+) patients because of high plasma glutamate level has been suggested to be a causative factor of low leukocyte reduced glutathione (GSH) level in these patients.[56,57]

TABLE 1. Biological properties of tocotrienol (1986–2000)

Biological Action	Year of Study	Description/Reference
Neuroprotective	2000	*Mouse*; protects against glutamate-induced neuronal death by suppressing inducible pp60 c-Src kinase activation[11]
Antiaging/antioxidant	2000	*C. elegans*; α-tocopherol acetate did not work[12]
Hypocholesterolemic, antioxidant, and antitumor	2000	*Chicken*; the number and position of methyl substituents in tocotrienols affect their hypocholesterolemic, antioxidant, and antitumor properties; tocotrienol better than α-tocopherol[13]
Antiproliferative and apoptotic	2000	*Mouse*; preneoplastic and neoplastic mammary epithelial cells: α- and γ-tocopherol had no effect on cell proliferation[14]
Modulating normal mammary gland growth, function, and remodeling	2000	*Mouse*; mammary epithelial cells more easily or preferentially took up tocotrienols as compared to tocopherols[15]
Anti-cancer (breast)	1999	*Human*; naturally occurring tocotrienols and RRR-delta-tocopherol are effective apoptotic inducers for human breast cancer cells[16]
ApoB level reduction in hypercholesterolemic subjects	1999	*Human*; in HepG2 cells it (not tocopherol) stimulates apoB degradation, possibly as the result of decreased apoB translocation into the endoplasmic reticulum lumen[17]
Lowering blood pressure; antioxidant	1999	*SHR*; supplement of γ-tocotrienol may prevent increased blood pressure, reduce lipid peroxides in plasma and blood vessels, and enhance total antioxidant status[18]
Anti-cancer	1999	*Human*; apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids[19]
Serum lipoproteins; platelet function	1999	*Human*; in men at risk for cardiovascular disease tocotrienol supplements had no marked favorable effects[20]
Serum triglycerides	1999	*Rat;* lower in tocotrienol fed; higher IgM productivity of spleen lymphocytes and IgA, IgG, and higher IgM productivity of mesenteric lymph node lymphocytes[21]
Immune function	1999	*Rats*; feeding affects proliferation and function of spleen and mesenetric lymph node lymphocytes[22]
Anti-cancer	1998	*Human*; inhibits the growth of human breast cancer cells irrespective of estrogen receptor status[23]
Transfer protein	1997	α-tocopherol transfer protein binds α-tocotrienol with 11% efficiency compared to a-tocopherol[24]
Anti-cancer	1997	*Human*; inhibited proliferation of estrogen receptor-negative MDA-MB-435 and -positive MCF-7 breast cancer cells[25] *Mouse*; isoprenoids suppress the growth of murine B16 melanomas *in vitro* and *in vivo*[26]

TABLE 1. (*continued*) Biological properties of tocotrienol (1986–2000)

Biological Action	Year of Study	Description/Reference
Lymphatic transport	1996	*Rat*; preferential absorption of alpha-tocotrienol compared to gamma- and delta-tocotrienols and alpha-tocopherol[27]
Antioxidant	1995	*Human*; controls the course of carotid atherosclerosis[28]
Hypocholesterolemic	1995	*Human*; lowered plasma cholesterol level in hypercholesterolemic subjects [29]
Anti-cancer	1995	*Human*; tocotrienol, not tocopherol, suppresses growth of a human breast cancer cell line in culture[30]
Antioxidant	1995	*Rat*; Protects brain against oxidative damage[31]
Hypocholesterolemic	1995	Isoprenoid-mediated suppression of mevalonate synthesis depletes tumor tissues of two intermediate products, farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are incorporated post-translationally into growth control-associated proteins[32]
Hypocholesterolemic	1994	*HepG2;* the farnesyl side chain and the methyl/hydroxy substitution pattern of gamma-tocotrienol responsible for HMG CoA reductase suppression[33]
Anti-cancer	1994	*Human;* suppresses activation of Epstein-Barr virus early antigen expression in PMA-activated lymphoblastoid Raji cells[34]
Hypocholesterolemic and antioxidant	1993	*Rat;* spares plasma tocopherol[35]
Antioxidant	1993	*In vitro;* tocotrienol is better than tocopherol; tocotrienol is located closer to the cell membrane surface[36]
Antioxidant	1993	*Human;* dietary tocotrienols become incorporated into circulating human lipoproteins where they react with peroxyl radicals as efficiently as the corresponding tocopherol isomers [7]
Anti-cancer	1993	*Rat*; tocotrienol. chemopreventive in hepatic tumor model[38]
Hypocholesterolemic	1993	Regulates cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase[39]
Hypocholesterolemic	1992	*In vitro*; posttranscriptional suppression of HMG-CoA reductase by a process distinct from other known inhibitors of cholesterol biosynthesis[40]
Anti-hypertensive	1992	*Rat*; depressed (better than α-tocopherol) age-related increase in the systolic blood pressure of spontaneously hypertensive rats[41]
Antioxidant regeneration	1992	*In vitro*; facilitates antioxidant recycling[42]
Antioxidant (lipid-phase)	1991	*In vitro*; better than α-tocopherol[43]

TABLE 1. (*continued*) Biological properties of tocotrienol (1986–2000)

Biological Action	Year of Study	Description/Reference
Hypocholesterolemic	1991	*Human*; lowered serum cholesterol in hypercholesterolemics[44]; lowered both serum total cholesterol (TC) and low-densitylipoprotein cholesterol[45] *Pigs;* reduced plasma cholesterol, apolipoprotein B, thromboxane B2, and platelet factor 4 in pigs with inherited hyperlipidemias [6]
Anti-cancer	1991	*Rat*; tocotrienol. chemopreventive in hepatic tumor model[47]
Anti-cancer	1991	*Rat*; tocotrienol, but not tocopherol, was chemopreventive in mammary tumor model [8]
Anti-cancer	1989	*Rat*; tocotrienol-rich palm oil prevented chemicallyinduced mammary tumorigenesis[49]
Anti-cancer	1989	*Mouse*; intraperitoneally injected tocotrienol prevented transplanted tumors[50]
Anti-cholesterol	1986	*Chicken*; three double bonds in the isoprenoid chain essential for the inhibition of cholesterogenesis; tocopherols do not share this property[51]

NOTE: SHR: spontaneously hypertensive rats; HMG CoA reductase: 3-hydroxy-3-methylglutaryl coenzyme A reductase.

Our initial interest was focused on the rescue of human lymphocytes from glutamate-induced oxidative damage, loss of thiols, and death.[58,59] We observed that several antioxidants are effective in preventing glutamate-induced lymphocyte toxicity.[54,58–60] Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by reactive oxygen species (ROS).[61] There are two forms of glutamate toxicity: receptor-initiated excitotoxicity[62] and non-receptor-mediated, glutamate-induced toxicity.[63] One model used to study oxidative stress–related neuronal death is to inhibit cystine uptake by exposing cells to high levels of glutamate.[64] The induction of oxidative stress by glutamate in this model has been demonstrated to be a primary cytotoxic mechanism in C6 glial cells,[65,66] PC-12 neuronal cells,[67,68] immature cortical neurons cells,[64] and oligodendroglial cells.[69] We were therefore led to investigate the role of oxidants and antioxidants in glutamate-induced death of cells of the nervous system.

Our first study in this direction was with C6 glial cells, where we noted that 0.1–1 mM lipoic acid or *N*-acetyl-L-cysteine was able to protect C6 glial cells against glutamate-induced death. This effect of these thiol antioxidants was mediated by their ability to increase cellular GSH levels.[66] Glutamate challenge to C6 cells was with associated increased accumulation of [ROS]i. Because the glutamate-induced cell death process was antioxidant-inhabitable and oxidant-associated, we sought to characterize the cellular events that are regulated by oxidants and antioxidants during the course of glutamate-induced death. HT neuronal cells, lacking a functional excitotoxic pathway, are commonly used to characterize the oxidative stress component of cell death.[63,70–81]

In HT4 cells,[82] we observed that glutamate challenge resulted in elevated $[ROS]_i$ and depleted $[GSH]_i$ and that the death process was inhibited in cells pretreated with several chemical classes of antioxidants.[83] We observed that compared to water-soluble antioxidants, lipophilic antioxidants were clearly more effective in preventing glutamate-induced death.[83] Our focus was therefore turned on the vitamin E family.[11] A comparison of the efficiency of α-tocopherol with α-tocotrienol to protect HT4 cells challenged with glutamate showed that tocotrienol was clearly more potent than tocopherol on a matched concentration basis.[11] The neuroprotective property of tocotrienol was clearly observed at a concentration of 50 nmol/L, and complete protection was achieved with 100 nmol/L even when tocotrienol was treated 2–3 hours after glutamate challenge. At these concentrations, tocopherol clearly failed to protect. After supplementation to humans, the level of α-tocotrienol in the plasma has been estimated to be 0.98 ± 0.8 mM.[84,85] Therefore, the neuroprotective effects of tocotrienol that we were observing corresponded to 1/10th of the concentration found in the plasma of supplemented humans. The observation that nanomolar concentrations of tocotrienol had neuroprotective properties was of outstanding interest. Particularly so because our observation constituted the first evidence that an antioxidant vitamin could have such potent cell regulatory properties at nanomolar concentrations. Later studies in our laboratory confirmed that the neuroprotective properties of nanomolar concentrations of tocotrienol were also applicable to primary fetal rat cortical neurons challenged with HCA or glutamate or even buthionine-S-R-sulfoximine.[86] Nanomolar concentrations of tocotrienol failed to protect against chemically generated peroxyl radicals. Micromolar concentration of tocotrienol was required for such protection.[11,86]

We were thus led to the conclusion that at nanomolar concentration, tocotrienol functions by an antioxidant-independent mechanism. Further study of the molecular processes suggested that nanomolar concentrations of tocotrienol potently regulate key signaling pathways involved in neuronal death. These findings constitute the first evidence establishing that trace amounts of tocotrienol may have potent signal transduction regulatory properties.[11]

MOLECULAR CHECKPOINTS IN NEURONAL CELL DEATH: OPPORTUNITIES FOR TOCOTRIENOL REGULATION

Executioners of cellular death in the nervous system are of diverse nature and are known to recruit a multitude of signaling pathways. It is not within the scope of this section to discuss such complexities. We undertake to focus on those specific mediators of neuronal death that have been identified by us to be tocotrienol sensitive in neuronal cells.[11,86]

pp60 c-Src

Our results indicate that inhibition of inducible pp60 c-Src kinase and extracellular signal responsive kinase activation represent a key mechanism by which nanomolar concentrations of tocotrienol protects glutamate-challenged HT4 cells.[11] In neurons and astrocytes, pp60 c-Src is present at levels 15–20 times higher than those found in fibroblasts. The specific activity of the c-Src protein from neuronal cultures

is 6- to 12-times higher than that from the astrocyte cultures, suggesting a key function of this protein in neurons.[87] Src family kinases are able to induce caspase-independent cytoplasmic events leading to cell death.[88] We noted that glutamate-induced death of HT4 neurons is not sensitive to caspase inhibitors.

Extracellular Signal Responsive Kinase

The extracellular signal responsive kinase (ERK) subfamily of mitogen-activated protein kinases (MAPKs) has been implicated in the regulation of cell growth and differentiation.[89] The role of ERK in neuronal degeneration is less clear and may depend upon the specific neuronal cell type. ERK activation is typically associated with cell survival, proliferation, and differentiation, given the activation by mitogens and some cell survival factors.[90–92] However, activation of ERK contributes to neuronal cell death in certain *in vitro* models of neurotoxicity.[93–96]

Recently we[11] and others[80] have demonstrated that sustained activation of ERK plays a central role in mediating glutamate-induced death of murine hippocampal HT cells. Consistent with these findings supporting the role of ERK in neuronal cell death, inhibition of MEK-1/2, the upstream activators of ERK, afforded some degree of protection against apoptosis generated by nerve growth factor withdrawal of differentiated PC12 cells.[97] MEK-1 inhibition also protects against neuron cell damage induced by focal cerebral ischemia in rats.[98]

Activation of ERK involves a two-step protein kinase cascade lying upstream from ERK, in which the Raf family are the MAP kinase kinase and the MEK1/MEK2 isoforms are the MAP kinases. The linear sequence of Raf MEK ERK constitutes a major component of the ERK cascade. Growth factor-regulated–receptor protein tyrosine kinases and G protein–coupled receptors represent two major modulators of inducible ERK function.[99] More recently, Src family tyrosine kinases have been identified to possess inducible ERK regulatory function.[100–103] Whether glutamate-induced activation of c-Src in HT4 cells directly or indirectly regulates ERK induction remains to be determined.

The Eicosanoid Pathway

Lowered $[GSH]_i$ and elevated lipid peroxidation represents one of the early cellular events after glutamate challenge. Reduced glutathione is a key survival factor in cells of the nervous system, and lowered $[GSH]_i$ is one of the early markers of neurotoxicity induced by a variety of agonists.[104,105] With the use of immature cortical neurons and HT cells, it has been shown that a decrease in $[GSH]_i$ triggers the activation of neuronal 12-lipoxygenase (12-LOX), which leads to the production of peroxides, the influx of Ca^{2+}, and ultimately to cell death.[72]

We have noted that in neuronal cells, a decrease in $[GSH]_i$ is not lethal *per se*, but that it may serve as a signal to activate mechanisms that signal for death.[86] Our observations bring to light that strategies to inhibit 12-LOX are able to rescue neuronal cells from glutamate-induced death. Given that Src kinase activity is also known to regulate inducible lipoxygenase activity,[106] it is difficult to delineate the relative contribution of lowered [GSH]i and activated Src in activating lipoxygenase in glutamate-treated cells. Of importance, metabolites of the lipoxygenase pathway are known to be able to activate ERK. Hydroxyeicosatetraenoic acid (HETE), a lipoxy-

genase metabolite of arachidonic acid (AA), leads to activation of Erk via the Raf-1/MEK signal transduction pathway.[107] More recently, 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE), a 12-LOX metabolite of AA, has been specifically shown to be responsible for ERK1/2 induction.[108]

Although our studies indicate that 12-LOX may play a central role in glutamate-induced death of HT4 cells, the source of substrate (that is, AA for 12-LOX) remains unidentified. AA can be produced from phospholipids by the actions of PL (phospholipase) A2, PLC, or PLD. Cleavage of phosphatidylcholine by PC-PLC yields diacylglycerol, which can give further rise to AA through the action of diacylglycerol lipase. Diacylglycerol, subject to negative regulation by vitamin E,[109] can signal for the activation of protein kinase C (PKC), and PKC modulates glutamate toxicity. Various neuropathological conditions appear to be associated with the activation of phospholipases, which release lipid metabolites that either are directly toxic to neurons or act as second messengers.[110,111] Phosphatidylcholine constitutes the majority of phospholipid in brain tissues, and it has been shown that PC-PLC inhibitor blocks glutamate toxicity in neuronal cells by uncoupling cystine uptake from glutamate inhibition, allowing the maintenance of glutathione synthesis and cell viability.[73] Exposure of synaptosomal membranes to phospholipases A2, C, and D results in their depolarization and an increase of the negative surface potential. In the case of phospholipases A2 and C, these changes are associated with a decrease of the microviscosity of the membrane lipid bilayer. Vitamin E has been shown to stabilize synaptosomal membranes against the damaging action of the phospholipases. This stabilization is caused by reconstitution of the transmembrane potential and an increase of microviscosity of the phospholipase-treated membranes.[112]

DIETARY TOCOTRIENOL IN THE BRAIN

The concern that dietary tocotrienol may not reach the brain[113] has substantially dampened general interest to investigate the neuroprotective properties of tocotrienol. In this context, it is important to note that tocotrienols are relatively unstable and may not be retained well when added to the laboratory chow. We have found that gavaging of tocotrienol suspended in vitamin E–deficient corn oil represents an effective approach of delivery of the vitamin to laboratory rodents. In our first study that looked at the tissue availability of tocotrienol, we fed rats a tocotrienol-rich fraction (TRF; 1.23 mmol α-tocotrienol and 0.94 mmol α-tocopherol per g weight) isolated from palm oil[15,23,30,114–116] provided by the Carotech Sdn Bhd of Malaysia. Eight-day pregnant rats were fed daily (intragastrically) with 1 g/kg body weight of TRF suspended in vitamin E–stripped corn oil for nine days. On day 17 of pregnancy, rats were killed and tissues collected from the mother and fetus. Vitamin E content in the whole brain was measured with the use of a 12-channel CouloArray high-performance liquid chromatography-ultraviolet (HPLC-UV) electrochemical system.[117] We observed that TRF feeding increased α-tocopherol and α-tocotrienol content in the maternal brain by 0.1-fold and 5-fold, respectively. Dietary TRF increased fetal brain α-tocotrienol content more than 20-fold. These results provide the first evidence that dietary tocotrienol does reach the brain. Subsequent studies with lower doses of tocotrienol (5 mg/kg body weight; Tocomin, Carotech, Malay-

sia) over a longer period of time support observations of our first study that dietary tocotrienol is clearly available to the brain.

VITAMIN E–SENSITIVE GENES IN THE BRAIN

We have determined that lowering vitamin E in the diet of pregnant mothers results in marked vitamin E deficiency in the fetal tissues. Using this system as a model, we sought to identify vitamin E–sensitive genes in the developing fetal brain.[118] The transcriptomes of developing fetal brains from E-sufficient and E-deficient groups were compared with the use of the U34A rat genome high-density oligonucleotide GeneChip array. This array analyzed approximately 7,000 full-length sequences and approximately 1,000 EST clusters. With the use of raw data from all replicates available from both groups, a total of six pair-wise comparisons were generated. The average (six pair-wise comparisons) fold-changes of all the genes that were differentially expressed were calculated.

Our results indicated that a majority of genes remained unchanged in response to dietary vitamin E deficiency. A total of 645 (7.3%) genes were upregulated in the E-sufficient compared to the E-deficient group. Of these candidates, the expression of 416 genes increased by a magnitude of 2-fold or more. On the other hand, 152 (1.7%) of the genes were downregulated with 74 of them lowered by 2-fold or more. With the use of the *t*-test analysis, a total 144 genes were observed to have changed significantly in the E-deficient group compared to the E-sufficient group. Next, genes for those in which the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. With the use of this approach to data analysis, a total of 19 probe sets were found to be upregulated and 34 repressed in the E-sufficient group compared to the E-deficient group.

Among the upregulated genes, two probe sets targeting hemeoxygenase-3 (HO-3) were increased by 3.9- and 3.1-fold, respectively. In contrast, the expression of maspin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), apolipoprotein B (apoB), and G protein beta1 subunit (rGb1) genes was highly (3- to 5-fold) repressed. HO-3 was one of the few E-sensitive genes upregulated in fetal brains. HO isozymes, HO-1, HO-2, and HO-3, are heat shock protein 32 protein cognates with a known function of catalyzing the isomer-specific oxidation of the heme molecule, including that of NO synthase. HO-1 is highly inducible, whereas HO-2 and HO-3 are constitutively expressed. These proteins play a central role in cellular defense mechanisms. HO activity is responsible for the production of equimolar amounts of CO, biliverdin, and free Fe. Recent findings with the HO suggest that these proteins may serve as an intracellular "sink" for NO.

LINE1 was identified to be another E-sensitive transcript. The long interspersed elements 1 (LINE-1) or L1 family of interspersed repeats accounts for at least 10% of the mammalian genome. Like other interspersed repeat DNA families in genomes of other organisms, L1 is dispersed and amplified throughout the genome by a series of duplicative transposition events. Because of the high copy number of L1 sequences in the genome, L1 is abundantly represented in the RNA population of most cells. However, most of the transcripts that contain L1 are the result of fortuitous transcription and are not intermediates in L1 retrotransposition. This high background of L1-

containing transcripts, many of which are truncated and rearranged, makes it difficult to distinguish the transcript encoded by an active L1 element(s).

ApoB mRNA was one of top candidates that were lower in the E-sufficient group compared to the E-deficient fetal brain. ApoB plays a central role in lipoprotein metabolism and exists in two isoforms in plasma, apoB-100 and apoB-48. High levels of apoB and LDL cholesterol have been associated with an increased risk for coronary heart disease. An earlier study has shown that administration of TRF (100 mg/day) decreases serum apoB. Tocopherol has been shown to inhibit PKC activity in cells.[119] PKC-regulated chloride channel was one of the genes suppressed in the E-sufficient fetal brain.[118]

SIGNIFICANCE

The critical significance of vitamin E in neurological health and disease was recognized several decades ago. Since then, vitamin E research has developed in a highly asymmetric fashion, with emphasis on α-tocopherol in particular, and with the least studied natural vitamin E being the tocotrienols. Tocotrienols are naturally occurring and are routinely consumed by humans with no documented adverse effects. Our work builds on the striking observation showing that trace amounts of tocotrienol possess potent neuroprotective properties. The observation is indeed "striking" because, as a general trend, nutrients are required at high micromolar or millimolar levels to influence biological responses. The neuroprotective ability of 100 nM (1/10th of the concentration achieved in the plasma of humans receiving supplement) tocotrienol may be viewed as the most potent of all properties of vitamin E characterized so far. Thus, a new frontier defining the molecular basis of vitamin E action is unfolding.

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