

An Insight of Vitamin E as Neuroprotective Agents

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Abstract: Nervous system is the network of nerve cells that transmits nerve impulses throughout the body. It is rich in both unsaturated fats and irons, making it predominantly susceptible to oxidative stress and damage. Oxidative stress reflects the disruption of the redox balance between the formation and clearance of highly free radicals, for instance reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative stress will further damage the cell lipid, protein and DNA. Oxidative stress has a role in the modulation of critical cellular functions, such as apoptosis program activation, ion transport and calcium mobilization which lead to cell death. Many studies were conducted to prevent neuronal cell death caused by oxidative stress through administration of free radical scavenging antioxidant, such as vitamin E. Vitamin E is known as a chain-breaking antioxidant that showed the capability to increase the viability of neuronal cells that had undergone glutamate injury by inhibiting glutamate-induced pp60 (c-Src) kinase activation. Vitamin E occurs in 8 forms, namely α -, β -, γ - and δ -tocopherols and α -, β -, γ - and δ -tocotrienols. Tocotrienols differ from tocopherols by possessing an unsaturated isoprenoid side chain instead of a saturated phytol tail. Tocotrienols, compared to tocopherols, are lightly studied due to the abundance of α -tocopherol in the human body and its antioxidant properties. Nevertheless, recent studies showed that α -tocotrienol is more effective in preventing lipid peroxidation compared to α -tocopherol. Furthermore, tocotrienol was discovered to protect neuronal cell through antioxidant-independent activities. The tocotrienol-rich fraction (TRF) is an extract that consists of 75% tocotrienol and 25% α -tocopherol. TRF was reported to possess potent antioxidant, anti-inflammation, anticancer and cholesterol-lowering properties. Thus, this writing highlights the significant neuroprotective effects of tocotrienol and tocopherol.

Keywords: neuroprotective agents; vitamin E; oxidative stress; tocotrienols; tocopherols.

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INTRODUCTION

Antioxidant

It known that aerobic organisms have developed a series of defense mechanisms, which involve antioxidants, in response to free radical production in order to maintain free radicals' level compatible with cellular functions and metabolic processes^[1]. Antioxidant defense mechanism can be classified into enzymatic and non-enzymatic. The enzymatic defense mechanism includes superoxide dismutase (SOD), GSH peroxidase (GPx) and catalase (CAT), whereas non-enzymatic antioxidant defenses include vitamin E, ascorbic acid (vitamin C), glutathione (GSH) and other antioxidants^[2].

GLUTATHIONE (GSH)

Glutathione (GSH) is the main thiol antioxidant and

redox buffer of the cell^[3,4]. It is a tripeptide comprised of glutamate, glycine and cysteine. It is synthesized in the cytosol by 2 enzymes that utilize ATP, that is GSH synthetase and gamma-glutamylcysteine (γ -GluCys) synthetase^[5]. The gamma-glutamylcysteine synthetase forms dipeptide gamma-glutamylcysteine by utilizing cysteine and glutamate as substrates. Gamma-glutamylcysteine is then merged with glycine in a reaction catalyzed by GSH synthetase thus forming GSH. GSH production is controlled by feedback inhibition of the γ -GluCys synthetase reaction by the end product GSH^[6]. Total GSH in the cells can be free or bound to protein. The free GSH is present in reduced form, which will be converted to the oxidized form (GSSG) during oxidative stress and can be restored to the reduced form by the action of glutathione reductase (GR)^[7]. The oxidation-reduction pathway of GSH is shown in Figure 1.

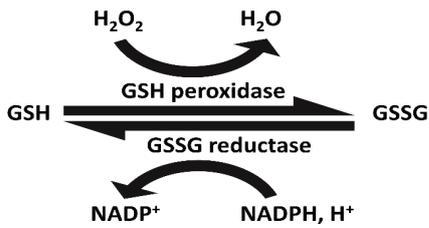


Figure 1. Oxidation-reduction pathway of reduced glutathione (GSH) and oxidized glutathione (GSSG).

GSH exerts its protection against oxidative stress by several approaches. Firstly, GSH directly scavenges reactive hydroxyl free radicals, ROS and radical centers on DNA and other biomolecules. Also, GSH functions as the cofactor for several detoxifying enzymes, for instance glutathione-S-transferases (GSTs) and glutathione peroxidase (GPx), which are vital in protection against oxidative stress. GPx is an enzymatic antioxidant which is predominantly responsible for the intracellular reduction of hydrogen peroxide (H₂O₂) to water with the support of GSH as electron donor^[8]. Thus, GSH is oxidized to glutathione disulfide (GSSG) through GPx activity and quickly restored to GSH by the reaction catalyzed by glutathione reductase (GR). Whereas, glutathione-S-transferases conjugate GSH to free radicals, for instance hydrogen peroxides, thus reducing the deleterious interactions between free radical and essential cellular components^[9,10]. Furthermore, GSH provides reducing capacity for the formation of deoxynucleotides by ribonucleotide reductase, the reduction of dehydroascorbate to ascorbate and restoration of vitamin E from radical form^[2,11].

Also GSH has been suggested to function as neuro-hormone based on the presence of extracellular GSH in brain, the release of GSH from brain slices upon stimulation, the specific binding of GSH to extracellular receptors and the induction of sodium currents in neocortex, and the stimulation of a signal cascade in astrocytes^[12,13]. Furthermore, some studies suggested that astrocytes support neuronal cells by means of providing GSH and cysteinylglycine (CysGly), which is the GSH precursor to neuronal cells^[6].

VITAMIN E

Vitamin E is a lipid-soluble vitamin vital for human nutrition and health. The term 'vitamin E' was first introduced by Evans and Bishop (1922)^[14] in describing a dietary factor in rat reproduction. The vitamin E family includes 8 different isomers, namely α -, β -, γ -, and δ -tocopherols and α -, β -, γ -, and δ -tocotrienols. Tocopherols and tocotrienols, as a group known as tocopherols comprise of a chromanol ring system and a polyprenyl side chain. The 8 isomers of vitamin E vary in the degree of antioxidant and biological activities. All tocopherols are amphipathic molecules, with the lipophilic isoprenoid side chain of tocopherol anchoring the membrane lipids, whereas the polar chromanol ring is exposed to the membrane surface^[15].

Vitamin E is the major component that present amongst the lipid elements of cell membranes and lipoproteins^[16]. Vitamin E is exclusively synthesized by photosynthetic eukaryotes and other oxygenic photosynthetic organisms for instance cyanobacteria. Therefore, vitamin E is ingested along with fat-containing food, like nut oil seeds, egg yolk, vegetable oils, margarine, soya bean, wheat, avocados and germ^[15].

Vitamin E has numerous biological functions. The pharmacologic use of vitamin E, in doses 10 to 50 times the daily requirement, was recommended in 1947 for the treatment of an array of cardiovascular disorders^[17]. The chain-breaking antioxidant properties of vitamin E was detected in the 1950s and consequently proved to be useful in preventing lipid peroxidation by scavenging chain-carrying peroxy radicals and generates an induction period^[18,19,20]. Furthermore, studies reported that severe vitamin E deficiency in human will leads to neuromuscular abnormalities because of free radical damage to the nerve cells^[21,22]. Vitamin E deficiency seldom occurs in human as a result of dietary deficiencies but occurs as a result of genetic abnormalities in the α -tocopherol transfer protein (α -TTP)^[23]. Vitamin E also possesses non-antioxidant functions, with vital role in cellular signaling by regulating protein kinase C^[24]. Moreover, vitamin E in combination with selenium were exhibiting ability to prevent loss of spermatogenesis in males^[25]. Some studies also indicated that vitamin E prevents most of the glutamate-induced neuronal cell death^[26]. Furthermore, dietary of vitamin E can enhance immune responses in numerous animal models^[27].

Metabolism of vitamin E

The hydrophobic nature of vitamin E make it preferentially located in oil storage organs, fat deposits and in cell membranes. It is transported around the body as an element of plasma lipoproteins. After ingestion of dietary vitamin E, it will be absorbed into the enterocyte, followed by packaging into chylomicrons. These nascent chylomicrons are then secreted into the lymphatic circulation. During the chylomicron catabolism in the circulation, the absorbed vitamin E is transferred to circulating lipoproteins and drained into the bloodstream. The high-density lipoprotein (HDL) in the bloodstream donates apolipoprotein C-II (APOCII) and apolipoprotein E (APOE) to the nascent chylomicron and thus converts it to a mature chylomicron. Lipoprotein lipase (LPL) is bound to the endothelial lining of capillary walls. During the lipolysis by LPL, various form of vitamin E could be transferred to tissues. Also, vitamin E could be exchanged between HDLs and other circulating lipoproteins, which could deliver vitamin E to the peripheral tissues. The resultant chylomicron remnant from lipolysis are primarily taken up by the liver through the chylomicron remnant receptors^[28]. In liver, remnant chylomicron-associated vitamin E is incorporated into nascent very-low density lipoproteins (VLDL) via the action of α -TTP^[29]. One of the vital determinants of vitamin E biological activity is the affinity of its analogues for α -TTP. The α -TTP has higher preference to α -tocopherol compared to other vitamin E isomers^[30]. When the VLDL are secreted into the plasma circulation, VLDL are converted into intermediate density lipoprotein (IDL) and low density

lipoprotein (LDL) via the action of LPL^[31,32]. Vitamin E is then transferred from plasma to cells through uptake facilitated by receptor-mediated lipoprotein endocytosis, lipid transfer proteins and lipases, and selective lipid uptake^[33]. Studies demonstrated that LDL receptor were facilitating the tissue incorporation of plasma vitamin E as part of LDL, while LPL and phospholipid transfer protein enable the tissue incorporation of plasma vitamin E as part of triglyceride-rich lipoprotein^[34–36]. Furthermore, IDL and LDL have LDL receptor-binding domains which allow receptor-mediated lipoprotein endocytosis to facilitate uptake of vitamin E into the peripheral tissue^[37]. The pathways of vitamin E absorption and distribution are depicted in Figure 2.

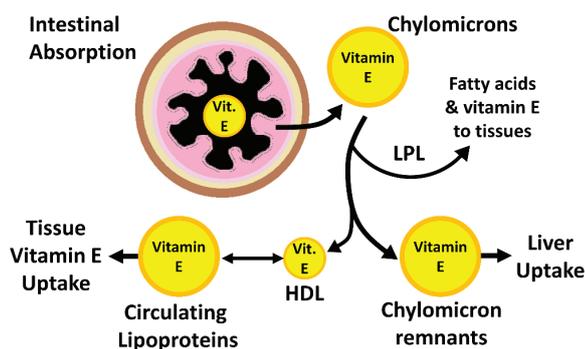


Figure 2. Pathways for vitamin E absorption and distribution.

Vitamin E is one of the most vital lipid-soluble antioxidants that protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction^[31]. As an antioxidant agent, vitamin E does not work independently in scavenging free radicals. It is a part of the redox antioxidant system. Vitamin E is efficiently reduced from its free radical form (tocotrienoxyl or tocopheroxyl) back to its reduced native form via enzymatic or non-enzymatic mechanisms. Vitamin C can directly restore vitamin E and thiol antioxidant, for instance GSH, and indirectly restore vitamin E via redox antioxidant network. This system maintains the concentration of vitamin E radicals low. Hence, the loss or consumption of vitamin E is prevented^[38].

ISOMER OF VITAMIN E

Tocopherol

Tocopherol contains a chromanol ring and a saturated phytyl side chain^[39]. The structural formulae of tocopherols are shown in Figure 3. Tocopherol is primarily found in sunflower and olive oils. Among 8 isomers of vitamin E, α -tocopherol was firstly derived from wheat germ oil and named in 1936 by Evan *et al.*^[40]. The α -tocopherol have the highest bioavailability among the isomers because of the recognition of α -TTP^[41]. The core function of α -tocopherol is terminating the chain reaction of lipid peroxidation to inhibit cell membrane and LDL from oxidative disintegration^[42]. Tocopherol also provides protection against peroxynitrite-induced lipid oxidation. Other than antioxidant function, vitamin E has functions in cell

signaling activities, for instance regulation of protein kinase C, inhibition of cyclooxygenase-2 activity and modulation of phospholipase A2 activity were due to the present of α -tocopherol. The α -tocopherol could dilate blood vessels and interferes with aggregation of platelets^[43]. Osakada *et al.*^[44] reported that 1–10 μM α -tocopherol effectively protects striatal neurons against cytotoxicity induced by a L-buthionine-S,R-sulfoximine (BSO) via the reduction of oxidative stress. Study indicated that α -tocopherol can effectively relieve neuronal damage induced by oxygen-centered free radicals^[45]. Also, α -tocopherol functions in regulating inflammation by reducing the release of cytokine interleukin-1 β (IL-1 β) via inhibition of 5-lipoxygenase pathway^[45].

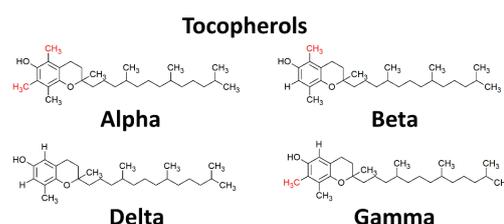


Figure 3. Structural formulae of Tocopherols.

Tocotrienol

Tocotrienols vary from tocopherols by having 3 double bonds in the hydrophobic tridecyl side chain^[42]. Figure 4 depicted the structural formulae of tocotrienols. Tocotrienols are rich in barley oil and palm oil. More than 95% of studies on vitamin E focusing on α -tocopherol due to its richness in the human body and its antioxidant functions. Nevertheless, recent studies exhibited that tocotrienol possesses health-promoting properties such as vital neuroprotective effect, cholesterol lowering and anti-cancer properties that are usually not displayed by tocopherols^[46]. Even though tocotrienols have low bioavailability, its antioxidant activity is higher than tocopherols^[47]. The α -tocotrienol exhibited better peroxy radical scavenging potency than α -tocopherol in liposomal membrane^[48]. The unsaturated side chain of tocotrienol allowing even distribution of tocotrienol in the membrane bilayer that further enhance the interaction of chromanol ring of α -tocotrienol with lipid radicals. Tocotrienols also moves between lipid vesicles much faster than α -tocopherol. Furthermore, the chromanoxyl radical of α -tocotrienol (α -tocotrienoxyl) was to be recycled in membranes and lipoproteins more rapidly compared to α -tocopheroxyl radical^[49,50].

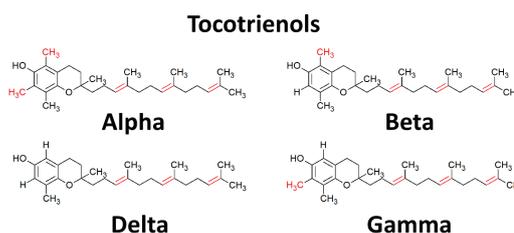


Figure 4. Structural formulae of Tocotrienols.

FUNCTIONS OF VITAMIN E

Antioxidant

Vitamin E efficiently inhibits lipid peroxidation and scavenges the chain-propagating peroxy radical. The scavenging outcome of α -tocotrienol was 1.5-fold higher than α -tocopherol in liposomes^[49]. Moreover, α -tocotrienol was 6.5 times more effective in protecting cytochrome P-450 against oxidative damage. The tocotrienol-rich fraction (TRF) from palm oil is significantly more effective than α -tocopherol in inhibiting oxidative damage in rat brain mitochondria induced by ascorbate- Fe^{2+} , the free radical initiator azobis (2-amidopropane) dihydrochloride (AAPH) and photosensitization^[51]. Furthermore, palm TRF at micromolar concentration providing better protection against copper-induced oxidation of plasma low density lipoprotein and also lipid peroxidation in human umbilical vein endothelial cells (HUVEC), as compared with α -tocopherol^[52]. Moreover, the efficacy of α -tocotrienol in protection against Fe^{2+} NADPH-induced lipid peroxidation in rat liver microsomes was 40 times higher than α -tocopherol^[49]. This could strongly suggest that α -tocotrienol has greater scavenging effect compared to α -tocopherol.

Neuroprotection

Recent studies demonstrated that vitamin E have health benefit properties which go beyond their known antioxidant activity. Studies indicated that α -tocotrienol prevented both oxidative stress-dependent and oxidative stress-independent apoptosis, whereas δ - and γ -tocotrienol only inhibited oxidative stress-dependent apoptosis. This displays that neuroprotective effect of α -tocotrienol could be mediated via non-antioxidant anti-apoptotic actions in addition to its antioxidant property^[53]. Moreover, nanomolar concentrations of α -tocotrienol could block glutamate-induced neuronal cell death, while α -tocopherol did not exhibit this property^[54]. Furthermore, nanomolar concentration of α -tocotrienol could protect glutamate-induced cell death in mouse neuroblastoma HT4 cell via inhibition of 12-lipoxygenase and phospholipase A2 activation that further interfere the state of phosphorylation^[26,54,55]. Additionally, tocotrienols effectively inhibited the activation of pp60 c-src kinase, a kinase that centrally involved in glutamate-induced cell death^[26]. For neuroprotection properties, studies reported that other sources (e.g. microbial resources) were also demonstrating strong antioxidants^[56-66] and neuroprotective properties^[67-72], for instance radical scavenging and metal chelating potentials^[73-80].

Other Beneficial Properties of Vitamin E

Numerous studies indicated that tocotrienols could suppress proliferation and induce apoptosis of several tumor cells such as breast, liver, lung, colon, stomach, skin, pancreas and prostate cancer cells^[81-87]. The γ -tocotrienol and δ -tocotrienol were reported to have anti-tumor activity in breast cancer cell irrespective of estrogen receptor status^[88,89]. The γ -tocotrienol also prevents cholesterol synthesis by suppressing

3-hydroxy-3-methylglutaryl-CoA reductase activity via a post-transcriptional mechanism^[90]. The cardioprotective effects of tocotrienol are also facilitated via their ability to suppress inflammation thus reduce the expression of adhesion molecules and monocyte-endothelial cell adhesion^[91].

BIOMARKER OF NEURONAL CELL INJURY

The continuous supply of oxygen and glucose is extremely important for brain energy metabolism. The disruption of this supply for a few minutes can introduce a sequence of biochemical events that lead to cell swelling, leakage and damage leading to neuronal cell death^[92]. Intracellular components, such as neuron specific enolase (NSE), can be detected in the extracellular fluid and cerebrospinal fluid (CSF) upon neuronal damage^[93]. Among various intracellular proteins, the concentrations of NSE, S100 β , glial fibrillary protein (GFAP) and myelin basic protein (MBP) exhibited positive correlation to the severity of the brain damage^[94]. The NSE catalyzes the conversion of 2-phospho-D glycerate to phosphoenolpyruvate in glycolytic pathway and localized predominantly in neuronal cytoplasm^[95]. The level of NSE in the cerebrospinal fluid has been used as markers of neuronal damage in patients with a variety of neurologic conditions including status epilepticus and metastatic lung cancer. Furthermore, positive correlation was reported between the glutamate-induced changes of the neuron-specific enolase efflux fraction^[96]. NSE is highly expressed as a glycolytic enzyme to replenish the ATP supply when energy depletion occurs, which could be due to neurotoxic agents for instance glutamate^[97]. Meanwhile, S100 β is a calcium-binding protein localized in astrocytes. The S100 β levels were increased after central nervous system lesions. Furthermore, high level of NSE and S100 β were reported in the CSF of infants and children after traumatic brain injury^[98].

CONCLUSION

Vitamin E, which made up of tocotrienols and tocopherol isomers, is a known chain-breaking antioxidant. Studies demonstrated that vitamin E have health benefit properties beyond their known antioxidant activity. With the α -tocotrienol preventing both oxidative stress-dependent and oxidative stress-independent apoptosis, while δ - and γ -tocotrienol only inhibited oxidative stress-dependent apoptosis. These findings demonstrated that neuroprotective effect of α -tocotrienol could be mediated via non-antioxidant anti-apoptotic actions in addition to its antioxidant property^[53]. Furthermore, TRF and α -tocopherol at concentration of 100 to 300 ng/mL demonstrated minor prophylactic properties but significant recovery ability in improving the glutamate-injured cell viabilities in both mono-culture and co-culture model. TRF at nanomolar concentration also exhibited better protection to neuronal cell against glutamate toxicity compared to α -tocopherol. Therefore, the putative mechanism of TRF and α -tocopherol action in protecting and recovering glutamate-injured cells was of great interest and warrant further research. More *in vivo* studies should be performed

to further understand the recovery mechanism of TRF and α -tocopherol in a complete body system.

Conflict of Interest

The authors declare that there is no conflict of interest in this work.

Authors Contributions

The literature review and manuscript writing were performed by H-MY and K-LL.

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