Protective Effect of Tocotrienol on In Vitro and In Vivo Models of Parkinson’s Disease

Tatsuya MATSURA
Division of Medical Biochemistry, Tottori University Faculty of Medicine, 86 Nishi-cho, Yonago 683–8503, Japan

Summary Parkinson’s disease (PD) is a common progressive neurodegenerative disease. It has been reported that oxidative stress contributes, at least in part, to its pathogenesis. Although dietary epidemiological studies suggest that sufficient intake of vitamin E may prevent the onset of PD, antioxidative therapy for PD with exogenous antioxidants involving α-tocopherol has not been successful in the clinical setting thus far. In recent years, the non-antioxidant activities of vitamin E have been given attention to. In the present study, to determine the antioxidant-independent cytoprotective activity of vitamin E, we investigated whether tocotrienols (T3s), another members of vitamin E family, exhibit the cytoprotective effect in cell and mouse models of PD independently of their antioxidant activities. Treatment with T3s, especially γ- and δ-T3s, exhibited cytoprotective effects via activation of PI3K/Akt signaling pathway in a cellular PD model. We also identified estrogen receptor (ER) β as an upstream mediator of PI3K/Akt signaling and demonstrated the direct binding of T3 to ERβ in vitro. Silencing expression of caveolin suppressed the cytoprotective effects of T3, indicating that caveola formation plays an important role in the cytoprotection by T3 via ERβ/PI3K/Akt signaling pathway. Thus it has been shown that T3 exerts cytoprotective function by a novel mechanism, which includes membrane ERβ/PI3K/Akt signaling via caveola formation as well as its antioxidant activity. Furthermore, we revealed that δ-T3 treatment relieved PD-related symptoms in PD model mice. These results suggest that T3 elicits the cytoprotective effects via ERβ/PI3K/Akt signaling pathway in cellular and murine PD models.

Key Words tocotrienol, Parkinson’s disease, estrogen receptor β, non-antioxidant activity, PI3K/Akt signaling, caveola

1. Non-Antioxidant Activities of Vitamin E

Vitamin E is a fat-soluble vitamin and includes two classes of tocopherol (Toc) and tocotrienol (T3). Tocs and T3s have respectively 4 derivatives (namely α, β, γ, and δ) according to the position and number of methyl groups on their chromanol rings (Fig. 1). Tocs are abundantly found in vegetable oils and almond. Palm oil, wheat germ and rice bran contain a high concentration of T3s. Vitamin E activity is generally considered to be mostly related to the antioxidant properties of the chemical structure. Free hydroxyl group on the chromanol ring is responsible to the antioxidant properties. However, recently non-antioxidant activities of vitamin E have received a lot of attention. These activities include modulation of signal transduction and gene expression, and alteration of membrane lipid domains. In most cases, non-antioxidant function of vitamin E is based on the experimental results that other antioxidants have no significant effects under the same conditions or vitamin E exhibits the beneficial effect without the reduction of lipid peroxide contents. It has been reported that α-Toc and γ-Toc do not function as the ligands of peroxisome proliferator-activated receptor γ (PPARγ) but upregulate the endogenous PPARγ ligand independently of their antioxidant activities, leading to the promotion of adiponectin gene expression (1). Another study has shown that α-Toc and γ-Toc directly bind protein kinase C (PKC) and regulate the activation of PKC (2). In regard to alteration of membrane lipid domains, in HER2-positive human breast cancer cells, HER2 receptors in lipid rafts dimerize and result in the autophosphorylation and activation of mitogenic signal transduction. However, in γ-T3-treated cells, γ-T3 accumulates in the lipid rafts and physically interferes with HER2 dimerization, resulting in the suppression of HER2 activation and subsequent HER2 oncogenic mitogenic signaling (3). In relation to non-antioxidant activities of vitamin E, we found a novel cytoprotective function of T3s mediated by the binding of T3s to the membrane receptor using in vitro and in vivo Parkinson’s disease (PD) models as will be described later (4, 5).

2. Parkinson’s Disease and α-Toc

Parkinson’s disease (PD) is a neurodegenerative disorder resulting in death of dopaminergic neurons in the substantia nigra (6). PD is recognized as the most common neurodegenerative disorder after Alzheimer’s disease. The incidence of PD ranges from 10 to 18 per 100,000 persons. Age is the greatest risk factor for the development of PD. Age of peak incidence is 80-years-
old and above. Gender is an established risk factor with a male-to-female ratio of approximately 3:2. Clinical manifestations of Parkinson’s disease include resting tremor, muscular rigidity, bradykinesia, and postural anomalies. In PD the following pathological hallmarks have been recognized: degeneration of dopaminergic neurons in the substantia nigra, and accumulation of Lewy bodies. Environmental factors such as oxidative stress, mitochondrial dysfunction, abnormality of protein degradation, and endoplasmic reticulum stress are implicated in the pathogenesis of PD. Genetic factors related to α-synuclein, parkin, DJ-1, etc. are also implicated. Given that oxidative stress is implicated in the pathogenesis of PD, antioxidants including vitamin E are potential candidates for neuroprotective therapy against PD. However, there are conflicting findings on vitamin E protection against PD. Some studies showed that intake of α-Toc reduced the risk of PD (7, 8). In contrast, certain study suggests that α-Toc does not affect the risk of PD (9). Moreover, DATATOP clinical study (10) showed no beneficial effect of α-Toc on PD progression.

3. Neuroprotective Effects of T3s in In Vitro and In Vivo PD Models That Are Independent of Their Antioxidant Activities

Therefore, we first examined whether vitamin E other than α-Toc, namely T3 exhibits neuroprotective effect in PD models, and then examined whether the effect is mediated by its antioxidant activity or another mechanisms. To examine the neuroprotective effect of T3, we used mitochondrial dysfunction-related PD model (4). We treated SH-SY5Y cells with MPP+ and/or T3, and at 48 h after treatment we measured cell viability. MPP+ reduced cell viability to around 40%. Simultaneous treatment with T3s significantly increased viability dose-dependently. Next, we examined whether T3s affect MPP+-induced oxidative stress. However, T3s did not significantly reduce the production of lipid hydroperoxides and protein carbonyl by MPP+. Next, we examined the effect of T3s on oxidative stress-independent PD-related neurotoxicities, that is, proteasome inhibitor or endoplasmic reticulum stressor-induced toxicities. T3s, especially γ- and δ-T3, also significantly protected cells against these neurotoxicities. These findings suggest that T3s protect cells independently of antioxidant activities. Thus, we hypothesized that the cytoprotective effect of T3s is dependent on intracellular signal transduction, and examined the activation of MAPK and PI3K/Akt pathways. Akt was phosphorylated at 120 min after T3 treatment, especially in response to γ-T3 and δ-T3. Next, as an index of Akt activation, we examined the translocation of Akt from the cytosolic area to the plasma membrane area. γ-T3 and δ-T3 induced translocation of Akt. These results suggest that γ-T3 and δ-T3 can activate the PI3K/Akt signaling pathway. Based on these results, we examined whether various kinds of kinase inhibitors can inhibit the cytoprotective effects of γ-T3 and δ-T3. PI3K inhibitors dramatically blocked T3-dependent cytoprotection. Our study has revealed that PI3K/Akt signaling is a key signal for T3-mediated cytoprotection. Since several receptors have been reported as upstream mediators of PI3K/Akt signaling in neuronal cells, we investigated the association of several receptors with T3-mediated cytoprotection by using various chemical inhibitors. Among inhibitors, estrogen receptor (ER) inhibitor, tamoxifen only inhibited the cytoprotective effect of T3. These results indicated that ER signaling is associated with cytoprotective effect of T3. Docking simulation study (11) has shown that δ-T3 can bind to ERβ. Therefore, we measured binding activity between T3s and ER using a radiometric competitive inhibition method. Direct binding of γ-T3 and δ-T3 to ERβ was shown. Next, we examined the translocation of ERβ under T3 treatment. In SH-SY5Y cells, ERβ localized mainly in extranuclear space such as cytosol and plasma membrane. Treatments with γ-T3 and δ-T3 induced ERβ translocation to perinuclear space. To clarify whether the cytoprotective effect of T3 is due to ERβ, we depleted ERβ using siRNAs. Knockdown of ERβ abrogated T3-mediated cytoprotection and PI3K/Akt

Fig. 1. Chemical structures of tocopherols and tocotrienols.
signal events triggered by γ-T3 treatment, we performed antibody array. Four of the 9 upregulated proteins were classified as endocytosis-related or actin/microtubules-related proteins. These findings suggest potential endocytic action such as caveola formation in γ-T3/ERβ signaling. Therefore, we depleted caveolin by using siRNAs. Knockdown of caveolin abrogated T3-mediated Akt phosphorylation. This finding suggests that PI3K/Akt activation may be an upstream mediator of caveola formation. Taken together, T3 binds to ERβ directly, and then ERβ signal induces activation of PI3K/Akt signal pathway, which triggers caveola formation including ERβ. Next, we examined ERβ-mediated neuroprotective effects of δ-T3 in a mouse PD model (5). We used MPTP mouse PD model. The neurotoxicant MPTP is metabolized by monoamine oxidase in astrocytes to generate the active metabolite MPP+ which was also used in in vitro model. MPP+ is taken up into dopaminergic neurons, resulting in the degeneration of dopaminergic neurons. MPTP was injected intraperitoneally 4 times at day 0, and oral administration of T3 was carried out everyday from day −2 to day 6. Tamoxifen was injected intraperitoneally every 2 days from day −2 to day 6. Wheel running activity and rotarod performance were measured on day 0, day 3, and day 7. Brain sampling was performed on day 7. We evaluated voluntary performance in wheel running activity. MPTP decreased the performance. δT3-treated MPTP mice showed a recovery of voluntary performance. Tamoxifen canceled the T3-induced recovery. Next, we evaluated motor coordination and balance using rotarod test. However, there was no significant difference of the time on a rotarod among the treatments. Immunohistochemistry showed a loss of dopaminergic neurons in the substantia nigra of MPTP-administered mouse brain. δT3 protected against MPTP-induced neurotoxicity. Tamoxifen canceled the positive effect of δT3. These results suggest that δT3 exhibits a neuroprotective effect via ERβ in a mouse PD model.

4. Conclusions
The results of the present study indicate that T3 elicits the cytoprotective effects via ERβ/PI3K/Akt signaling pathway in cellular and murine PD models.

Disclosure of State of COI
The author declares no conflicts of interest.

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