Biochemistry and Biophysics Reports 28 (2021) 101131

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

The effect of α-tocopherol, α- and γ-tocotrienols on amyloid-β aggregation and disaggregation in vitro

Nor Faeizah Ibrahim [a], Hamizah Shahirah Hamezah [b], Daijiro Yanagisawa [c,*], Mayumi Tsuji [d], Yuji Kiuchi [d], Kenjiro Ono [e], Ikuo Tooyama [c,**]

[a] Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Cheras, Kuala Lumpur, 56000, Malaysia
[b] Institute of Systems Biology (INBIOSIS), Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia
[c] Molecular Neuroscience Research Center, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, 520-2192, Japan
[d] Department of Pharmacology, Showa University School of Medicine, Shinagawa-ku, Tokyo, 142-8555, Japan
[e] Department of Neurology, Showa University School of Medicine, Shinagawa-ku, Tokyo, 142-8666, Japan

ARTICLE INFO

Keywords:
Amyloid-β
Tocotrienol
Tocopherol
Vitamin E
Alzheimer's disease

ABSTRACT

One of the neuropathological hallmarks of Alzheimer's disease (AD)—causing neurodegeneration and consequent memory deterioration, and eventually, cognitive decline—is amyloid-β (Aβ) aggregation forming amyloid plaques. Our previous study showed the potential of a tocotrienol-rich fraction—a mixture of naturally occurring of vitamin E analogs—to inhibit Aβ aggregation and restore cognitive function in an AD mouse model. The current study examined the effect of three vitamin E analogs—α-tocopherol (α-TOC), α-tocotrienol (α-T3), and γ-tocotrienol (γ-T3)—on Aβ aggregation, disaggregation, and oligomerization in vitro. Thioflavin T (ThT) assay showed α-T3 reduced Aβ aggregation at 10 μM concentration. Furthermore, both α-T3 and γ-T3 demonstrated Aβ disaggregation, as shown by the reduction of ThT fluorescence. However, α-TOC showed no significant effect. We confirmed the results for ThT assays with scanning electron microscopy imaging. Further investigation in photo-induced cross-linking of unmodified protein assay indicated a reduction in Aβ oligomerization by γ-T3. The present study thus revealed the individual effect of each tocotrienol analog in reducing Aβ aggregation and oligomerization as well as disaggregating preformed fibrils.

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia in the elderly, is a progressive neurodegenerative disorder that affects the memory and cognitive function of an individual. The two neuropathological hallmarks of AD are the accumulation of amyloid-β (Aβ) peptides to form senile plaques, and hyperphosphorylation of tau protein to form neurofibrillary tangles. Both events lead to synaptic and neuronal dysfunction and—eventually—to impaired cognitive performance [1]. The imbalance between production of Aβ in the brain and its clearance causes accumulation and eventually, AD pathogenesis, to occur [2]. Aβ fibrillization begins with the polymerization of soluble Aβ oligomers to form Aβ fibrils and plaques [3]. The accumulation of Aβ oligomers to form Aβ fibrils is driven by the interaction between aromatic residues of Aβ peptides [4]. Studies have shown that small aromatic molecules such as polyphenols can inhibit this interaction [5].

In a previous study, our group [6] revealed that tocotrienol-rich fraction (TRF) inhibited the formation of Aβ fibrils and Aβ oligomers in vitro. TRF is a natural mixture of vitamin E analogs derived from the palm oil of the *Elaeis guineensis* tree. It comprises two vitamin E series: tocotrienol (α-, β-, γ-, and δ-T3) and α-tocopherol (α-TOC). The series are distinguished by the presence of three double bonds at the side chain of the tocotrienol structure (Fig. 1). Vitamin E has shown beneficial effects in reducing the progression of degenerative diseases such as AD [7]. Many studies have identified vitamin E as an excellent antioxidant, anti-aging, and anti-inflammatory compound [8].

Our earlier findings provide insights into the effects of TRF on AD—particularly, via modulating amyloid pathology. However, the identity of the vitamin E analog in TRF exerting a better neuroprotective effect in terms of reducing amyloid pathology in vitro and in vivo—and hence, contributing to an improved cognitive function—remains unknown. A study by Khanna et al. [9] revealed that α-T3 at a nanomolar

* Corresponding author.
** Corresponding author.
E-mail addresses: daijiroy@belle.shiga-med.ac.jp (D. Yanagisawa), kinchan@belle.shiga-med.ac.jp (I. Tooyama).

https://doi.org/10.1016/j.bbrep.2021.101131
Received 8 July 2021; Received in revised form 7 September 2021; Accepted 7 September 2021

concentration provides more potent neuroprotection than that provided by α-TOC. However, no studies have investigated its effect on Aβ aggregation and disaggregation in vitro. Our current study therefore examined the effect of individual vitamin E analogs—particularly α-TOC, α-T3, and γ-T3—on Aβ aggregation and disaggregation in vitro.

2. Materials and methods

2.1. Preparation of α-TOC, α-T3, and γ-T3

We purchased α-TOC and α-T3 from Sigma-Aldrich (St. Louis, MO, USA), and γ-T3 from Cayman Chemical (Ann Arbor, Michigan, USA). We prepared a stock solution of 10 mM by dissolving it in ethanol and kept it at −30 °C until use. We carried out further dilutions using phosphate-buffered saline (PBS; Nacalai Tesque, Kyoto, Japan).

2.2. Preparation of Aβ42 aggregates

We prepared human Aβ1-42 peptide (Aβ42; Peptide Institute, Osaka, Japan) as described elsewhere [6]. To examine the effects of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation, we mixed Aβ42, individually, with α-TOC, α-T3, and γ-T3 to a final concentration of 10 μM Aβ42 plus 10, 30, 100, or 300 μM α-TOC, α-T3, or γ-T3. We used PBS containing ethanol at 0.3% or 3% as a control. We incubated the mixtures at 37 °C for 5, 10, and 24 h.

We further investigated the disaggregation effect of α-TOC, α-T3, and γ-T3 on Aβ42 fibrils preformed by the incubation at 37 °C for 3 h. We individually added α-TOC, α-T3, and γ-T3 to the Aβ42 fibrils to reach a final concentration of 10 μM Aβ42 plus 10, 30, 100, and 300 μM α-TOC, α-T3, and γ-T3, and incubated the mixtures at 37 °C for 5, 10, and 22 h.

2.3. Thioflavin T fluorescence assay

We conducted the ThT fluorescence assay as described previously [6]. We measured the fluorescence intensity in 96-well black plates (Greiner Bio-one, Frickenhausen, Germany) using an Infinite M200 microplate reader (Tecan, Grödig, Austria) at excitation and emission wavelengths of 450 nm and 482 nm, respectively. As a control, we mixed ThT with PBS containing only α-TOC, α-T3, or γ-T3. The Aβ42-specific fluorescence was calculated by subtracting the fluorescence intensity of the control solution. Each measurement was performed in duplicate. Data represent the means of three independent experiments.

2.4. Scanning electron microscopy (SEM) imaging

We observed the effect of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation and disaggregation with SEM imaging. We carried out sample preparation according to a previous study with modification [10]. Briefly, we spotted the reaction mixture onto a 150 mesh Formvar-coated nickel grid (Electron Microscopy Sciences, Hatfield, PA, USA) and dried for 20 min at room temperature. Then, we displaced the sample with 2.5% v/v glutaraldehyde in water for 5 min. We then washed the grid by dipping it in water 5 times, repeating the washing step 3 times. Next, we stained the grid with 2% filtered uranyl acetate in water for 30 s, further washed it 3 times in water, and allowed it to dry at room temperature. We observed samples using a field emission SEM (JEOL JSM-7505FA; JEOL, Peabody, MA, USA) at 25 kV. We selected the images of SEM randomly from the 150 mesh Formvar-coated nickel grid that was used to place the reaction mixtures, at a magnification of 25000X.

2.5. Photo-induced cross-linking of unmodified protein (PICUP) assay

We conducted the PICUP assay as described in a previous study [6]. Briefly, we mixed 50 μM Aβ40, Aβ42 peptides or

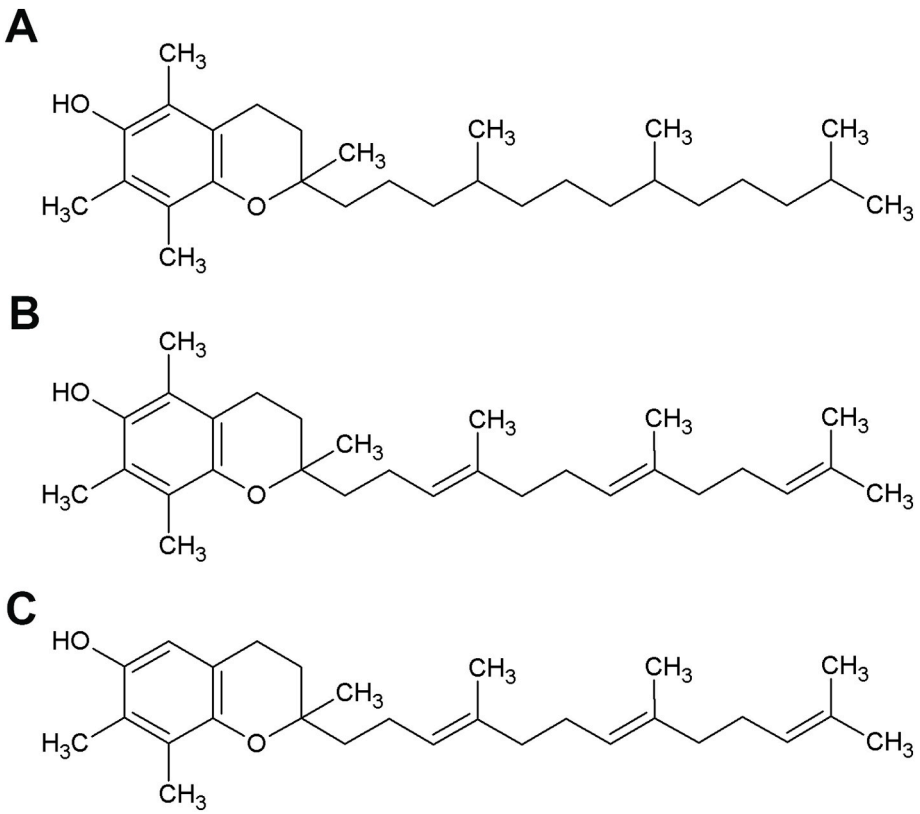

Fig. 1. Structure of tocopherol and tocotrienols: (A) α-tocopherol structure with no double bond at the side chain; (B) α-tocotrienol, and (C) γ-tocotrienol structures with three double bonds at the side chain.

glutathione-S-transferase (GST; Sigma-Aldrich, St. Louis, MO, USA), 2 mM tris (2,2′-bipyridyl) dichlororuthenium (II), and 40 mM ammonium persulfate with α-TOC, α-T3, and γ-T3 at a 1:1 ratio. Immediately, we cross-linked the mixture by PICUP. We subjected the samples to SDS-polyacrylamide gel electrophoresis under reducing conditions with 15%–20% Tricine gel (SuperSep Ace; Wako) and visualized the protein by silver staining with a SilverQuest Staining Kit (Invitrogen, California, USA).

2.6. Statistical analysis

We performed statistical analysis using Graphpad Prism 7 (GraphPad Software; La Jolla, CA, USA). We present data as mean ± standard error of the mean (S.E.M.). We compared group means by one-way analysis of variance followed by the Bonferroni post hoc test for multiple comparisons.

3. Results

3.1. Effect of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation

We performed a ThT assay to determine the effect of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation. We incubated 10-μM Aβ42 with 10, 30, 100, and 300 μM α-TOC, α-T3, or γ-T3 at 37 °C for 5 h, and then carried out the ThT fluorescence measurement. We regarded the average of the ThT fluorescence intensity in Aβ42 without compound as 100% (Fig. 2A–D). In a mixture of Aβ42 with 300 μM α-TOC, we noted a reduction of about 30% in the fluorescence percentage compared to that found in Aβ42 without compound. However, at 10 μM, the fluorescence percentage in α-T3 started to decrease significantly by about 20%, holding its significance at 30 μM, and at 100 μM and 300 μM concentrations, decreased further to about 30% that of Aβ42 without compound. Compared to Aβ42 without compound, γ-T3 showed a significant decrease at 10 μM and 300 μM. Furthermore, the fluorescence percentage of 10 μM γ-T3 decreased significantly by about 20%–30%, compared to that of α-TOC. We also investigated the effect of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation at different incubation times (Fig. 2E–H). We performed ThT fluorescence measurement directly after mixing 30 μM α-TOC, α-T3, or γ-T3 with 10 μM Aβ42 (Fig. 2E). In the absence of α-TOC, α-T3, or γ-T3, the relative fluorescence intensity of ThT in 10 μM Aβ42 increased progressively (Fig. 2F–H). However, we observed a significant reduction in the relative fluorescence intensity of ThT in Aβ42 in the presence, individually, of 30 μM α-TOC, α-T3, or γ-T3 compared to that found in Aβ42 without compound. At 10 and 24 h of incubation time, α-TOC reduced the relative fluorescence intensity. However, at 5, 10, and 24 h of incubation, α-T3 and γ-T3 significantly reduced the relative fluorescence intensity of ThT in Aβ42. We chose an incubation time of 5 h during which to assess the effect of different concentrations, individually, of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation, as above.

Next, we observed the effects, individually, of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation with scanning electron microscopy (SEM) imaging.

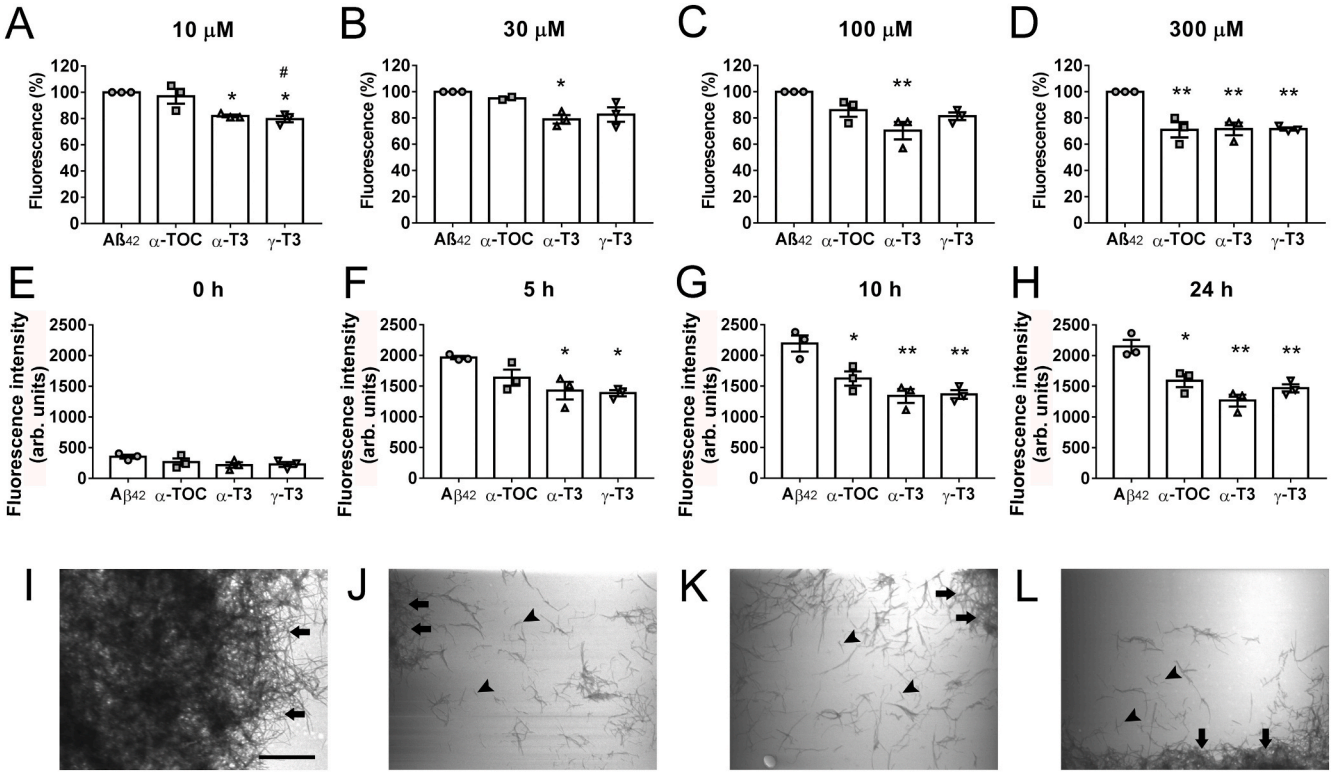

Fig. 2. Effect of α-TOC, α-T3, and γ-T3 on Aβ42 aggregation. (A–D) We mixed a final concentration of 10 μM Aβ42 samples with 10, 30, 100, or 300 μM α-TOC, α-T3, or γ-T3, incubated at 37 °C for 5 h before performing a thioflavin T (ThT) assay. (A) At 10 μM α-T3 and γ-T3 we observed about 20% reduction in the fluorescence percentage compared to that in Aβ42 incubated without compound. We found a significant difference in the fluorescence percentage reduction of γ-T3 and that of α-TOC. (B, C) α-T3 at 30 and 100 μM significantly reduced the fluorescence percentage by about 20%–30%. (D) α-TOC, α-T3, and γ-T3 significantly reduced the fluorescence percentage compared with that in Aβ42 incubated without compound. (E–H) We incubated Aβ42 at 37 °C with α-TOC, α-T3, and γ-T3, each at a final concentration of 10 μM and 30 μM respectively, and performed the ThT assay at 0, 5, 10, and 24 h. (E) We detected no difference in the relative fluorescence intensity at 0 h, (F) a significant difference for α-T3 and γ-T3 incubated at 5 h, (G, H) and a reduction in the relative fluorescence intensity for α-TOC, α-T3, and γ-T3 at 10 and 24 h of incubation time. We present data as mean ± S.E.M. Our results were significant at *$p < 0.05$, **$p < 0.01$ versus Aβ42 incubated without compound; and #$p < 0.05$ versus α-TOC. Electron microscopic images of Aβ42 incubated with and without α-TOC, α-T3, and γ-T3. (I) Ten-micromolar Aβ42 without compound incubated at 37 °C for 24 h. Ten-micromolar Aβ42 + 30 μM (J) α-TOC, (K) α-T3, and (L) γ-T3 incubated at 37 °C for 24 h. Arrows and arrowheads indicate Aβ fibrils and their sheared fragments, respectively. Scale bar: 1 μm.

For 10-μM Aβ42 incubated without α-TOC, α-T3, or γ-T3 at 37 °C for 5 h, we observed numerous fibrils (Fig. 2I). We made a similar observation (arrow) for 10 μM Aβ42 incubated with 30 μM α-TOC (Fig. 2J), α-T3 (Fig. 2K), or γ-T3 (Fig. 2L). However, we observed many short and sheared fragments (arrowhead) in α-TOC, α-T3, and γ-T3 mixtures compared to Aβ42 without compound.

3.2. *Effect of α-TOC, α-T3, and γ-T3 on disaggregation of preformed Aβ42 fibrils*

We determined Aβ42 fibrillization by incubation of 20 μM Aβ42 at 37 °C and performed a ThT assay at 0, 1, 3, 8, 13, and 24 h (Fig. 3A). We noted maximum measurements of ThT fluorescence intensity at 3 h of incubation, at which point the measurement remained consistent at a plateau until 24 h. We used 20-μM Aβ42 incubated for 3 h to determine the effect, individually of α-TOC, α-T3, and γ-T3 on Aβ42 disaggregation. We mixed α-TOC, α-T3, and γ-T3 at 10, 30, 100, and 300 μM with

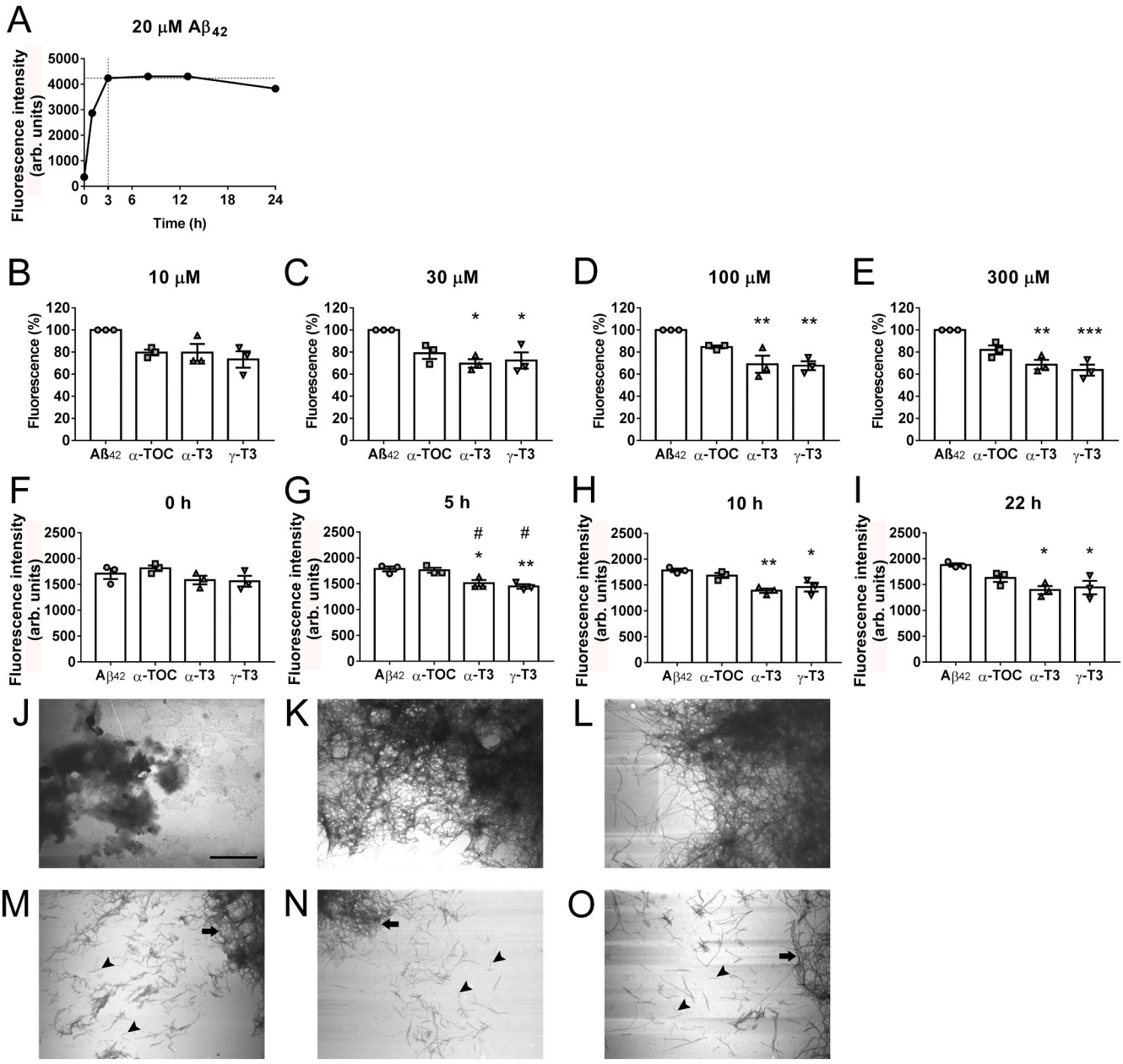

Fig. 3. Effect of α-TOC, α-T3, and γ-T3 on disaggregation of preformed Aβ42 fibrils. (A) We incubated 20 μM Aβ42 at 37 °C and monitored fibrillization with ThT assay at 0, 1, 3, 8, 13, and 24 h. (B–E) We incubated Aβ42 with α-TOC, α-T3, and γ-T3 for 5 h and carried out a relative ThT fluorescence intensity measurement. We found that α-T3 and γ-T3—but not α-TOC—significantly reduced the fluorescence percentage by about 30% compared to the reduction achieved after incubation of Aβ42 without compound. (F–I) We incubated Aβ42 with 30 μM α-TOC, α-T3, and γ-T3 and performed the relative ThT fluorescence intensity measurement at 0, 5, 10, and 22 h. α-T3 and γ-T3—but not α TOC—significantly decreased the relative ThT fluorescence intensity, compared to that for Aβ42 without compound. We present data as mean ± S.E.M, with significance at *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus Aβ42 incubated without compound; and #$p < 0.05$ versus α-TOC. Electron microscopic images of Aβ42 fibrils incubated with and without α-TOC, α-T3, and γ-T3. We incubated 20 μM Aβ42 at 37 °C for (J) 0 h, (K) 3 h, and (L) 25 h. At 3 h of Aβ42 incubation, we added 30 μM (M) α-TOC, (N) α-T3, and (O) γ-T3 and further incubated it for 22 h. We observed distinct Aβ42 fibrils (arrow) and also many short, sheared fragments (arrowheads). Scale bar: 1 μm.

Aβ42 fibrils (at a final concentration of 10 μM) and further incubated them at 37 °C for 5 h before performing the ThT measurement. We regarded the average ThT fluorescence intensity in Aβ42 without compound as 100% (Fig. 3B–E). We did not detect a significant decrease in the fluorescence percentage in α-TOC incubated with Aβ42 compared to Aβ42 incubated without compound. However, we observed a significant reduction of about 30% in the fluorescence percentage of α-T3 and γ-T3 incubated with Aβ42 at 30 μM, further reduced at 100 μM, consistent at 300 μM for α-T3, and further reduced at 300 μM for γ-T3, compared to that observed for Aβ42 incubated without compound. We further investigated the effect of α-TOC, α-T3, and γ-T3 on Aβ42 disaggregation at different incubation times (Fig. 3F–I). We found no difference in the relative fluorescence intensity of ThT in Aβ42 with or without compound (Fig. 3F). This indicated that the compounds showed no effect to the binding capability of ThT. We mixed 30-μM α-TOC, α-T3, and γ-T3 with 10 μM Aβ42 fibrils, and conducted ThT measurements before (0 h) and after incubation at 37 °C for 5, 10, and 22 h. At 0 h, we observed no difference in ThT relative fluorescence intensity in Aβ42 incubated with or without compounds. The relative fluorescence intensity in Aβ42 incubated without compound remained constant throughout the incubation periods. We found the relative fluorescence intensity of Aβ42 with α-T3 and γ-T3, on the other hand, to decrease at 5, 10, and 22 h incubation. Conversely, we did not detect a decrease in relative fluorescence intensity for α-TOC.

Next, we observed the mixtures with SEM to verify the effect of α-TOC, α-T3, and γ-T3 on Aβ42 disaggregation. We prepared 20-μM of Aβ42 and subjected the sample to SEM imaging before and after incubation at 37 °C. At 3 h of incubation, we observed distinct fibril morphologies as compared to those at 0 h (Fig. 3J and K). We made a similar observation for Aβ42 incubated without compound for another 22 h (Fig. 3L). We also found fibril morphologies in Aβ42 incubated with α-TOC, α-T3, and γ-T3 (arrows; Fig. 3M – O); however, we distinguished many short and sheared fragments (arrowhead) from Aβ42 incubated without compound (Fig. 3L).

3.3. Effect of α-TOC, α-T3, and γ-T3 on PICUP-Induced Aβ oligomers

We investigated the effect of α-TOC, α-T3, and γ-T3 on Aβ oligomerization by PICUP assay. In Fig. 4A we show the uncross-linked mixtures of 25 μM Aβ40 samples appearing as one band representing the monomeric form of Aβ40. The cross-linked mixtures of 25 μM Aβ40 without compound (control) resulted in the formation of dimers and trimers. A cross-linked mixture containing 25 μM Aβ40 with 30, 100, or 300 μM of α-TOC or α-T3 did not affect oligomer formations. However, cross-linked mixtures of 25 μM Aβ40 with 30 or 100 μM of γ-T3 showed a slight reduction in trimer formation. Also, a 300 μM γ-T3 mixture with 25 μM Aβ40 totally inhibited oligomerization. Similarly, cross-linked mixtures containing 25 μM Aβ42 with 30 or 100 μM of γ-T3 reduced the formation of pentamers, as observed in Fig. 4B. Remarkably, we observed complete inhibition in a mixture containing 25 μM Aβ42 with 300 μM γ-T3. We also conducted the PICUP experiment using a mixture of glutathione-S-transferase (GST) with α-TOC, α-T3, or γ-T3 at different concentrations. In Fig. 4C, we reveal the absence of an inhibitory effect of α-TOC, α-T3, or γ-T3 on GST oligomer formation. This finding thus confirmed the specificity of α-TOC, α-T3, and γ-T3 in the reaction with Aβ oligomerization.

4. Discussion

In the present study, α-TOC, α-T3, and γ-T3 showed the inhibition of Aβ aggregation and the promotion of Aβ disaggregation. The concentration in the present study was chosen based on the study by Yang et al. suggesting 30 and 300 μM of α-tocopherol quinine, a major derivative of α-TOC, modulate Aβ aggregation and disaggregation in vitro [10]. The activities of α-TOC and γ-T3 appeared at only a very high molecular ratio (30:1), compared with α-tocopherol quinine (3:1) [10] and curcumin derivatives (3:1) [11], suggesting the effects of α-TOC, α-T3, and γ-T3 on Aβ aggregation and disaggregation are low. This finding suggests the therapeutic effect of TRF on AD pathology in a mouse model of AD we reported in the previous study might be due to multiple effects of TOC and T3s in TRF on not only Aβ aggregation and disaggregation but also

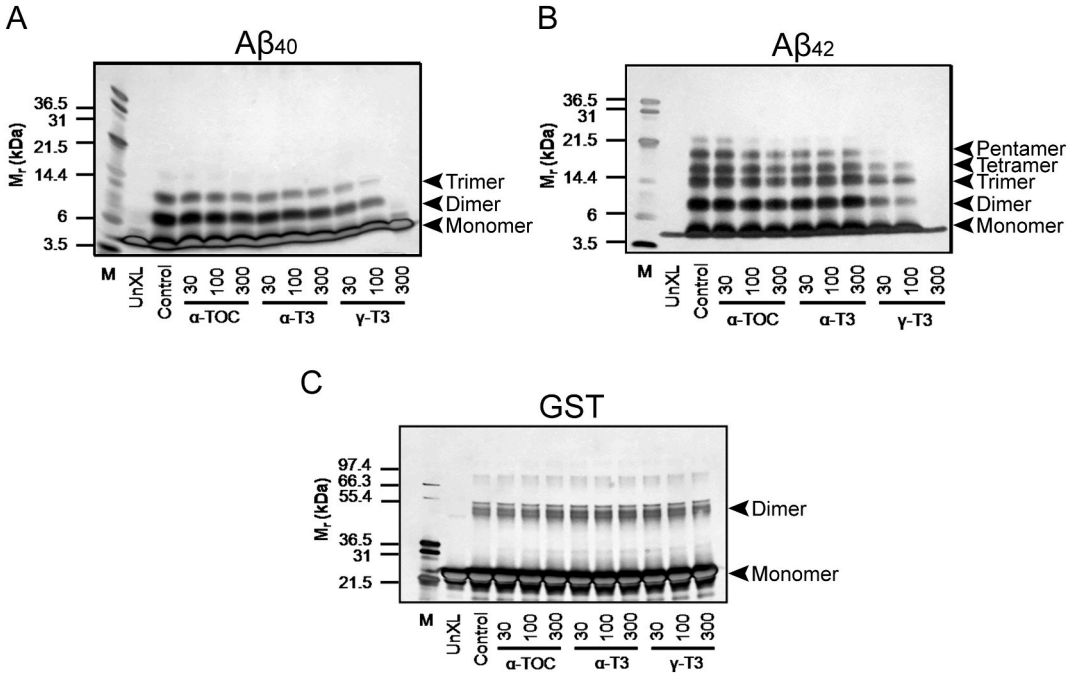

Fig. 4. Effect of α-TOC, α-T3, and γ-T3 on Aβ42 oligomerization. We mixed Aβ40 (A) and Aβ42 (B) peptides with phosphate-buffered saline (control), α-TOC, α-T3, and γ-T3 and cross-linked them in a PICUP assay. We subjected the samples to gel electrophoresis and visualized them by silver staining. While α-TOC and α-T3 showed no effects on Aβ oligomer formation, γ-T3 reduced oligomer formation of Aβ40 and Aβ42 in a dose-dependent manner. Neither α-TOC, α-T3, nor γ-T3 had any effect on GST oligomer formation (C). M: marker, UnXL: uncross-linked Aβ peptides prepared without light exposure.

others such as anti-oxidative stress, anti-inflammatory, and neuroprotection [8]. We speculate that these compounds reduced Aβ aggregation by directly binding to Aβ peptides and interfering with the structural conversion on the amyloid species. Previous studies using the other typical organic Aβ inhibitors such as curcumin and resveratrol postulated the capability of polyphenols to inhibit Aβ aggregation via the presence of aromatic structures [12,13]. Several studies have reported the significant role of the aromatic interactions in amyloid formation, as well as the self-assembly of complex supramolecular structures [14,15]. Since both tocopherol and tocotrienol comprise the aromatic ring in their structures, α-TOC, α-T3, and γ-T3 are likely to prevent the growth of Aβ aggregates via the interaction of these compounds with aromatic residues of Aβ to form π–π stacking arrangements between them.

We found that only α-T3 and γ-T3—but importantly, not α-TOC—were able to disaggregate preformed Aβ42 fibrils. This is in agreement with previous studies by Yatin et al. and Yang et al. demonstrating the inability of α-TOC at 50 μM and 300 μM, respectively, to inhibit Aβ fibril formation [10,16]. However, the effect of tocotrienol in modulating Aβ fibril formation was not investigated in these studies. To our knowledge, the present study is the first to report the direct effect of individual tocotrienol analogs (α-T3 and γ-T3) on Aβ aggregation and fibril formation in vitro. Vitamin E includes two groups of fat-soluble compounds—namely, tocopherol and tocotrienol, both of which share structural similarities which can be viewed as comprising a chroman head with two rings (one phenolic and one heterocyclic) and a phytyl tail. The presence of three double bonds at the C-3′, C-7′, and C-11' positions of the hydrocarbon tail distinguishes tocotrienol from tocopherol. Several lines of evidence suggest that determination of differential biopotencies exhibited by individual vitamin E isomers are influenced by the level of phytyl chain saturation and/or chroman ring methylation [17]. We therefore suggest that the chroman head in the structure of vitamin E may contribute to the inhibitory effect on Aβ aggregation, while the unsaturated side chain may contribute to the disaggregation of Aβ fibrils.

The present study did not investigate the effectiveness of γ-tocopherol (γ-TOC) to modulate Aβ aggregation, disaggregation and oligomerization. A previous study by Pahrudin Arrozi et al. revealed the treatment of SH-SY5Y-APP Swe with γ-TOC in vitro reduced Aβ42 levels, mitochondrial reactive oxygen species, and elevated ATP level compared to control [18]. Meanwhile, the study of a randomized trial by Morris et al. using postmortem human brains demonstrated the association between concentration of γ-TOC with lower amyloid load and neurofibrillary tangles severity [19]. The study also showed that higher α-TOC levels were associated with increased amyloid load except when the levels of γ-TOC were also high, at which point higher levels of α-TOC were associated with lower amyloid levels, suggesting that γ-TOC was able to modify the association of α-TOC with amyloid load [19]. These findings presented the important roles of γ-TOC in the modulation of amyloid pathology and therefore, future studies should consider the contribution of γ-TOC in AD neuropathology.

The Aβ oligomer has been considered the most toxic form of Aβ to inhibit hippocampal long-term potentiation and disrupt synaptic plasticity [2]. It was found to be elevated in the AD brain and the results were correlated with cognitive decline as measured by the Mini-Mental State Examination [20]. Therefore, a promising target for treating AD would be the inhibition of Aβ oligomerization. Our previous study found that TRF significantly inhibited Aβ oligomerization in vitro [6]. TRF is a mixture of α-TOC (168.0 mg/g), α-T3 (196.0 mg/g), β-T3 (24.0 mg/g), γ-T3 (255.0 mg/g), and δ-T3 (75.0 mg/g), which contains the highest amount of γ-T3 analog. TRF has been proved to modulate the expression of various proteins involved in the AD pathway—including amyloid beta A4 (APP) protein in the brain [21]—hence, contributing to the improved cognitive function in APP/PS1 mice [22]. A study by Umeda et al. [23] reported that rifampicin at a molar ratio of 4:1 showed complete inhibition of Aβ oligomer formation in PICUP assay. In the present study, a 1.2:1 and 4:1 M ratio of γ-T3 to Aβ reduced pentamer oligomer formation, and at a 12:1 M ratio, we observed a complete oligomer inhibition. We therefore identified γ-T3 as the most effective vitamin E compound to inhibit Aβ oligomerization. Gamma-T3 lacks one methyl group at the C-5′ position of the chromanol ring, distinguishing it from the other two compounds (α-TOC and α-T3) used in the study. The absence of the methyl group at the C-5' position of the chromanol ring is thus a key structure in exerting an inhibitory effect on Aβ oligomerization.

In conclusion, we found that α-TOC reduced Aβ aggregation at high concentrations. However, α-T3 reduced Aβ aggregation and also disaggregated preformed fibrils even at low concentrations. Moreover, γ-T3 showed remarkable effects as it reduced Aβ aggregation, disaggregated preformed fibrils, and also reduced Aβ oligomerization. Overall, each vitamin E analog showed different effects on Aβ aggregation, disaggregation, and oligomerization. Findings from the present study may therefore provide a better understanding of the potential role of the tocotrienols in the development of therapeutic agents for AD.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by JSPS KAKENHI, grant number JP26290022 (I.T.) from the Japan Society for Promotion of Science. The authors thank Mr. Takefumi Yamamoto for his excellent assisting with scanning electron microscope viewing. The authors would like to thank Enago (www.enago.jp) for the English language review.

References

[1] D.J. Selkoe, The molecular pathology of Alzheimer's disease, Neuron 6 (1991) 487–498, https://doi.org/10.1016/0896-6273(91)90052-2.
[2] D.J. Selkoe, J. Hardy, The amyloid hypothesis of Alzheimer's disease at 25 years, EMBO Mol. Med. 8 (2016) 595–608, https://doi.org/10.15252/emmm.201606210.
[3] L.C. Serpell, Alzheimer's amyloid fibrils: structure and assembly, Biochim. Biophys. Acta 1502 (2000) 16–30, https://doi.org/10.1016/s0925-4439(00)00029-6.
[4] O.S. Makin, E. Atkins, P. Sikorski, J. Johansson, L.C. Serpell, Molecular basis for amyloid fibril formation and stability, Proc. Natl. Acad. Sci. U. S. A 102 (2005) 315–320, https://doi.org/10.1073/pnas.0406847102.
[5] K. Ono, Y. Yoshiike, A. Takashima, K. Hasegawa, H. Naiki, M. Yamada, Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease, J. Neurochem. 87 (2003) 172–181, https://doi.org/10.1046/j.1471-4159.2003.01976.x.
[6] N.F. Ibrahim, D. Yanagisawa, L.W. Durani, H.S. Hamezah, H.A. Damanhuri, W. Z. Wan Ngah, M. Tsuji, Y. Kiuchi, K. Ono, I. Tooyama, Tocotrienol-rich fraction modulates amyloid pathology and improves cognitive function in AβPP/PS1 mice, J. Alzheim. Dis. 55 (2017) 597–612, https://doi.org/10.3233/JAD-160685.
[7] J. Frank, X.W.D. Chin, C. Schrader, G.P. Eckert, G. Rimbach, Do tocotrienols have potential as neuroprotective dietary factors? Ageing Res. Rev. 11 (2012) 163–180, https://doi.org/10.1016/j.arr.2011.06.006.
[8] C.K. Sen, C. Rink, S. Khanna, Palm oil–derived natural vitamin E α-tocotrienol in brain health and disease, J. Am. Coll. Nutr. 29 (2010) 314S–323S. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3065441/.
[9] S. Khanna, S. Roy, N.L. Parinandi, M. Maurer, C.K. Sen, Characterization of the potent neuroprotective properties of the natural vitamin E alpha-tocotrienol, J. Neurochem. 98 (2006) 1474–1486, https://doi.org/10.1111/j.1471-4159.2006.04000.x.
[10] S. Yang, W. Wang, T. Ling, Y. Feng, X. Du, X. Zhang, X. Sun, M. Zhao, D. Xue, Y. Yang, R. Liu, α-Tocopherol quinone inhibits β-amyloid aggregation and cytotoxicity, disaggregates preformed fibrils and decreases the production of reactive oxygen species, NO and inflammatory cytokines, Neurochem. Int. 57 (2010) 914–922, https://doi.org/10.1016/j.neuint.2010.09.011.
[11] D. Yanagisawa, H. Taguchi, S. Morikawa, T. Kato, K. Hirao, N. Shirai, I. Tooyama, Novel curcumin derivatives as potent inhibitors of amyloid β aggregation, Biochem. Biophys. Reports. 4 (2015) 357–368, https://doi.org/10.1016/j.bbrep.2015.10.009.
[12] B.Y. Feng, B.H. Toyama, H. Wille, D.W. Colby, S.R. Collins, B.C.H. May, S. B. Prusiner, J. Weissman, B.K. Shoichet, Small-molecule aggregates inhibit amyloid polymerization, Nat. Chem. Biol. 4 (2008) 197–199, https://doi.org/10.1038/nchembio.65.

[13] C.I. Stains, K. Mondal, I. Ghosh, Molecules that target beta-amyloid, ChemMedChem 2 (2007) 1674–1692, https://doi.org/10.1002/cmdc.200700140.
[14] J.J. Balbach, Y. Ishii, O.N. Antzutkin, R.D. Leapman, N.W. Rizzo, F. Dyda, J. Reed, R. Tycko, Amyloid fibril formation by A beta 16-22, a seven-residue fragment of the Alzheimer's beta-amyloid peptide, and structural characterization by solid state NMR, Biochemistry 39 (2000) 13748–13759, https://doi.org/10.1021/bi0011330.
[15] C.-J. Tsai, J. Zheng, R. Nussinov, Designing a nanotube using naturally occurring protein building blocks, PLoS Comput. Biol. 2 (2006), https://doi.org/10.1371/journal.pcbi.0020042 e42–e42.
[16] S.M. Yatin, S. Varadarajan, D.A. Butterfield, Vitamin E prevents alzheimer's amyloid beta-peptide (1-42)-induced neuronal protein oxidation and reactive oxygen species production, J. Alzheimers. Dis. 2 (2000) 123–131, https://doi.org/10.3233/jad-2000-2212.
[17] C.K. Sen, S. Khanna, S. Roy, L. Packer, Molecular basis of vitamin E action. Tocotrienol potently inhibits glutamate-induced pp60(c-Src) kinase activation and death of HT4 neuronal cells, J. Biol. Chem. 275 (2000) 13049–13055, https://doi.org/10.1074/jbc.275.17.13049.
[18] A. Pahrudin Arrozi, S.N.S. Shukri, W.Z. Wan Ngah, Y.A. Mohd Yusof, M.H. Ahmad Damanhuri, F. Jaafar, S. Makpol, Comparative effects of alpha- and gamma-tocopherol on mitochondrial functions in alzheimer's disease in vitro model, Sci. Rep. 10 (2020) 8962, https://doi.org/10.1038/s41598-020-65570-4.
[19] M.C. Morris, J.A. Schneider, H. Li, C.C. Tangney, S. Nag, D.A. Bennett, W.G. Honer, L.L. Barnes, Brain tocopherols related to Alzheimer's disease neuropathology in humans, Alzheimer's Dementia 11 (2015) 32–39, https://doi.org/10.1016/j.jalz.2013.12.015.
[20] J.L. Tomic, A. Pensalfini, E. Head, C.G. Glabe, Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction, Neurobiol. Dis. 35 (2009) 352–358, https://doi.org/10.1016/j.nbd.2009.05.024.
[21] H.S. Hamezah, L.W. Durani, D. Yanagisawa, N.F. Ibrahim, W.M. Aizat, S. Makpol, W.Z. Wan Ngah, H.A. Damanhuri, I. Tooyama, Modulation of proteome profile in AβPP/PS1 mice hippocampus, medial prefrontal cortex, and striatum by palm oil derived tocotrienol-rich fraction, J. Alzheim. Dis. 72 (2019) 229–246, https://doi.org/10.3233/JAD-181171.
[22] L.W. Durani, H.S. Hamezah, N.F. Ibrahim, D. Yanagisawa, M.L. Nasaruddin, M. Mori, K.A. Azizan, H.A. Damanhuri, S. Makpol, W.Z. Wan Ngah, I. Tooyama, Tocotrienol-rich fraction of palm oil improves behavioral impairments and regulates metabolic pathways in AβPP/PS1 mice, J. Alzheimers. Dis. 64 (2018) 249–267, https://doi.org/10.3233/JAD-170880.
[23] T. Umeda, K. Ono, A. Sakai, M. Yamashita, M. Mizuguchi, W.L. Klein, M. Yamada, H. Mori, T. Tomiyama, Rifampicin is a candidate preventive medicine against amyloid-β and tau oligomers, Brain 139 (2016) 1568–1586, https://doi.org/10.1093/brain/aww042.