

Therapeutic Potential and Anti-Amyloidosis Mechanisms of Tert-Butylhydroquinone for Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a major cause of dementia in the elderly with no effective treatment. Accumulation of amyloid- β peptide (A β) in the brain, one of the pathological features of AD, is considered to be a central disease-causing and disease-promoting event in AD. In this study, we showed that feeding male A β PP/PS1 transgenic mice, a well established mouse model of AD, with a diet containing phenolic antioxidant tert-butylhydroquinone (TBHQ) dramatically reduced brain A β load with no significant effect on the amounts of alpha- and beta-C-terminal fragments or full-length A β PP. Further studies showed that TBHQ diet inhibited the expression of plasminogen activator inhibitor-1 (PAI-1), a protease inhibitor which plays a critical role in brain A β accumulation in AD, accompanied by increases in the activities of tissue type and urokinase type plasminogen activators (tPA and uPA) as well as plasmin. Moreover, we showed that TBHQ diet increased the expression of low density lipoprotein related protein-1, a multi ligand endocytotic receptor involved in transporting A β out of the brain, and plasma A β_{40} and A β_{42} levels. We also showed that TBHQ diet increased the concentration of glutathione, an important antioxidant, and suppressed the expression of NADPH oxidase 2 as well as lipid peroxidation. Collectively, our data suggest that TBHQ may have therapeutic potential for AD by increasing brain antioxidant capacity/reducing oxidative stress level and by stimulating A β degradation/clearance pathways.

Keywords: Alzheimer's disease, amyloid- β degradation, amyloid- β efflux, antioxidant

INTRODUCTION

Alzheimer's disease (AD), a major course of dementia in the elderly, is a progressive neurodegenerative disease that gradually damages the neocortex and hippocampus. Despite extensive studies, there is no

effective treatment for this devastating disease due to an incomplete understanding of the etiology and pathophysiology of the disease. Formation of extracellular senile plaques, which are abnormal aggregates of amyloid- β peptide (A β), is a pathological feature of AD. Accumulation of both soluble and insoluble A β in the brain has been suggested to be a disease-causing and disease-promoting event in AD. Consequently, developing the strategies to reduce brain A β levels has been a focus of many studies including several

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clinical trials. Nonetheless, although several studies have shown that reduction of brain A β burden is associated with improvement of learning/memory function in animals and in AD patients [1–5], no drugs have been developed yet due to potential safety concerns or adverse effects.

A β production is increased in early-onset (familial) AD due to mutations in the amyloid- β protein precursor (A β PP) gene or the presenilin 1 and presenilin 2 (PS1 and PS2) genes. However, the mechanism underlying increased A β accumulation in the brain of late-onset (sporadic) AD, which account for 95% of the cases, remains largely unknown. The brain is a site with high metabolic activity, high concentrations of prooxidants such as iron, and low antioxidant capacity, and therefore is highly sensitive to oxidative damage. It has been well documented that oxidative stress increases with age and in AD. It has also been shown that oxidative stress contributes importantly to AD pathology including neuronal cell death and A β accumulation while antioxidants, including lipoic acid and N-acetylcysteine (NAC), reduce brain A β burden and/or improve cognitive function in animal models of AD [6–9]. These observations suggest therapeutic potential of antioxidants for the treatment of AD.

The phenolic compound tert-butyl hydroquinone (TBHQ) is a highly effective preservative used in a wide range of food and cosmetic products at the concentrations as high as 1% w/w with relative low toxicity (WHO Food Additives Series 4). In addition to directly scavenging free radicals [10], studies from this and other labs have shown that TBHQ increases the expression of the enzymes involved in the synthesis of glutathione (GSH), the most abundant intracellular free thiol and an important antioxidant, in various types of cells and in animal models [11–15]. TBHQ has also been shown to induce the expression of several other enzymes involved in detoxification and antioxidant defense through activation of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor controlling a pleiotropic cellular defense [14, 16, 17]. Most importantly, it has been reported that TBHQ protects neurons from oxidative damage induced by different toxicants or ischemia-reperfusion *in vitro* and *in vivo* [14, 15, 18–23]. Whether TBHQ can ameliorate pathological changes in AD, a disease with many pathological features of oxidative stress, however, has not been reported.

In this study, we explored the therapeutic potential of TBHQ for AD using A β PP/PS1 mice, a well-established animal model of AD. Our results showed that feeding A β PP/PS1 mice with TBHQ-

containing diet significantly reduced brain A β load. Further studies suggest that TBHQ does so probably by increasing antioxidant capacity/reducing oxidative stress and stimulating A β degradation and/or efflux from the brain. The results from this study suggest that TBHQ may have therapeutic value for AD.

MATERIALS AND METHODS

Animals and TBHQ feeding

A β PP/PS1 double transgenic mice, purchased from JAXMICE, were generated by co-injection of human A β PP and PS1 transgene constructs containing AD mutations (a mutant human presenilin 1 (DeltaE9) and a chimeric mouse/human amyloid- β protein precursor) and were maintained on a C57BL/6 genetic background. Two and a half month old male A β PP/PS1 mice were fed with either a control diet or 1% TBHQ-containing diet for 6 weeks. All the mice were maintained on a 12-h light/dark cycle at 22°C with free access to water and food. Body weight and food intake were recorded weekly in order to monitor potential toxicity of TBHQ. By the end of feeding, mice were euthanized and blood withdrawn from the heart, followed by transcardial perfusion with cold PBS as we have described before [24]. Brain was dissected sagittally into right and left hemispheres with the right hemisphere fixed in 10% PBS buffered formalin for immunostaining analysis and the left hemisphere dissected and hippocampus and cerebral cortex frozen in liquid nitrogen immediately for biochemistry analyses. All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

ELISA analyses of A β and PAI-1 proteins in the brain

For the measurement of soluble and insoluble A β , mouse brain tissues were prepared as we have described previously [24]. Briefly, the brain tissues were homogenized in a A β extraction buffer containing 20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 1% Triton X-100, 2% SDS, and protease inhibitors (complete protease inhibitor cocktail, Boehringer Mannheim, Mannheim, Germany), and centrifuged at 100,000 \times g for 1 h. Supernatant was collected (SDS soluble) and stored at -80°C until analysis. Pellets were dissolved in 70% formic acid (FA), gentle shaking at room temperature for 1 h, and then centrifuged at 100,000 \times g for 1 h. The supernatants were collected

(SDS insoluble/FA soluble). ELISA was performed to quantify SDS soluble and SDS insoluble/FA soluble A β using the A β ₄₀ or A β ₄₂ ELISA kits from Covance (Emeryville, CA) [24]. The FA extraction solution was neutralized and diluted 20 times with a neutralizing buffer containing 1 M Tris, 0.5 M Na₂HPO₄, and 0.05% NaN₃ before analysis by ELISA. For the measurement of PAI-1 protein in the brain, tissues were homogenized in Tris-HCl buffer containing 0.1% Triton-X 100, pH 8.5. After ultracentrifugation at 100,000 \times g for 1 h, the supernatants were used for the measurement of total PAI-1 protein levels using an ELISA kit as we have described before [26]. The results were calculated based on the standard curves and expressed as per protein concentrations.

Immunohistochemical staining of A β deposits and low density lipoprotein related protein-1 (LRP-1) expression in mouse brain

Immunohistochemical staining of A β deposits in the brain was conducted using monoclonal anti-human A β antibody 6E10 (Covance, Emeryville, CA) as previously described [24]. The amyloid load in the cerebral cortex and hippocampus was quantified by determining the percentage of the total section positively labeled by A β antibody using Axiovision automatic measurement software (Zeiss, Germany). For detection of the expression of LRP-1 protein in brain microvessels anti mouse LRP-1 light chain monoclonal antibody (5A6, Calbiochem) was used. The number of positive vessels was counted in five fields within cerebral cortex per mouse and the results were expressed as percentage of total number of blood vessels in the same area of the brain [25].

Western analysis

Mouse brain tissues were homogenized in a tissue extraction buffer containing 2% SDS and different protease inhibitors as described above. Western analyses of full-length A β PP and alpha/beta C-terminal fragments (α -/ β -CTFs) were performed using 4–20% gradient gel (Invitrogen). Full-length A β PP proteins were detected with 6E10 antibody (Covance, Emeryville, CA) while α - and β -CTFs were determined using rabbit polyclonal antibody CT695 against the C terminus of A β PP (Invitrogen). LRP-1 protein was detected using anti mouse LRP-1 light chain monoclonal antibody (5A6) while the protein of NADPH oxidase 2 (Nox2), an important producer of reactive oxygen species (ROS), was detected with a polyclonal

antibody (Santa Cruz, SC5827). Semi-quantification of the bands was performed by densitometric scanning using Image J software and normalized by β -actin. For detection of 4-hydroxy-2-nonenal (4-HNE) modified proteins, the brain tissues were homogenized and westerns run using non-reducing buffers. 4-HNE modified proteins were revealed by anti-4-HNE antibody (alpha Diagnostic, HNE-11 S).

Zymographic analysis of the activities of tissue type and urokinase type plasminogen activators

The activities of tissue type and urokinase type plasminogen activators (tPA and uPA, respectively) in mouse brain tissue were determined by zymographic analysis according to our previously described protocol [24, 26]. Briefly, equal amounts of proteins were loaded onto 12% SDS polyacrylamide gel containing 1% nonfat dry milk and 5 μ g/ml plasminogen. After electrophoresis, the enzyme reactions were initiated by incubating the gel in 0.1 M glycine-NaOH (pH 8.3) at 37°C for 16 h. The lytic bands (tPA and uPA activity) were revealed by Coomassie blue staining and quantified using Image J system. Gels without plasminogen were also run simultaneously to ensure that the lytic bands are due to plasminogen activators. The tPA and uPA bands were identified based on both molecular weight and the loss of lytic activity upon inclusion of the plasmin inhibitor aprotinin (2 μ g/ml).

Measurement of plasmin activity in brain tissue

Plasmin activities were measured using a specific chromogenic substrate Tosyl-glycyl-prolyl-lysine-4-nitrilide-acetate (Chromzyme PL, from Roche Applied Sciences) as described before [24]. The reaction mixture contained 33 mM Tris (pH 8.2), 6.4 mM NaCl, and 0.5 mM Chromozym PL. The time-dependent production of 4-nitraniline was followed by monitoring the absorbance at 405 nm. Plasmin activity was calculated using the 4-nitraniline extinction coefficient ($\epsilon_{405 \text{ nm}} = 1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and expressed as unites per protein concentration.

Measurement of glutathione content in brain tissue

Brain tissues were sonicated in 5% sulfosalicylic acid and centrifuged. GSH concentrations in the supernatant were measured by a redox cycle assay as described before [27]. The protein content was measured by a BCA kit and GSH concentrations were calculated based on standard curve run together with

the samples. The results were expressed as nmol/mg protein.

Statistical analysis

Data were expressed as mean \pm SEM and evaluated by one-way ANOVA. Statistical significance was determined by Fisher LSD test wherein $p < 0.05$ were considered statistically significant.

RESULTS

TBHQ diet caused no obvious toxicities in A β PP/PS1 mice

Two and a half month old male A β PP/PS1 mice were fed with a diet containing 1% TBHQ for 6 weeks. The body weight and food intake were monitored during TBHQ feeding. No significant changes in body weight

or food intake were observed in TBHQ fed mice as compared with control diet fed mice (data not shown). There was also no significant difference in the mortalities between TBHQ diet and control diet fed mice (data not shown). The results indicate that TBHQ, under the current feeding conditions, causes no significant toxicity.

Effects of TBHQ diet on brain A β load in A β PP/PS1 mice

A β load in the brain was analyzed by immunohistochemical staining and ELISA techniques. Immunohistochemical staining showed that TBHQ feeding significantly reduced the numbers of senile plaque deposits in cerebral cortex/hippocampal region in A β PP/PS1 mice as compared to control diet fed mice (Fig. 1A, B). ELISA data further showed that TBHQ feeding significantly reduced the levels of both SDS

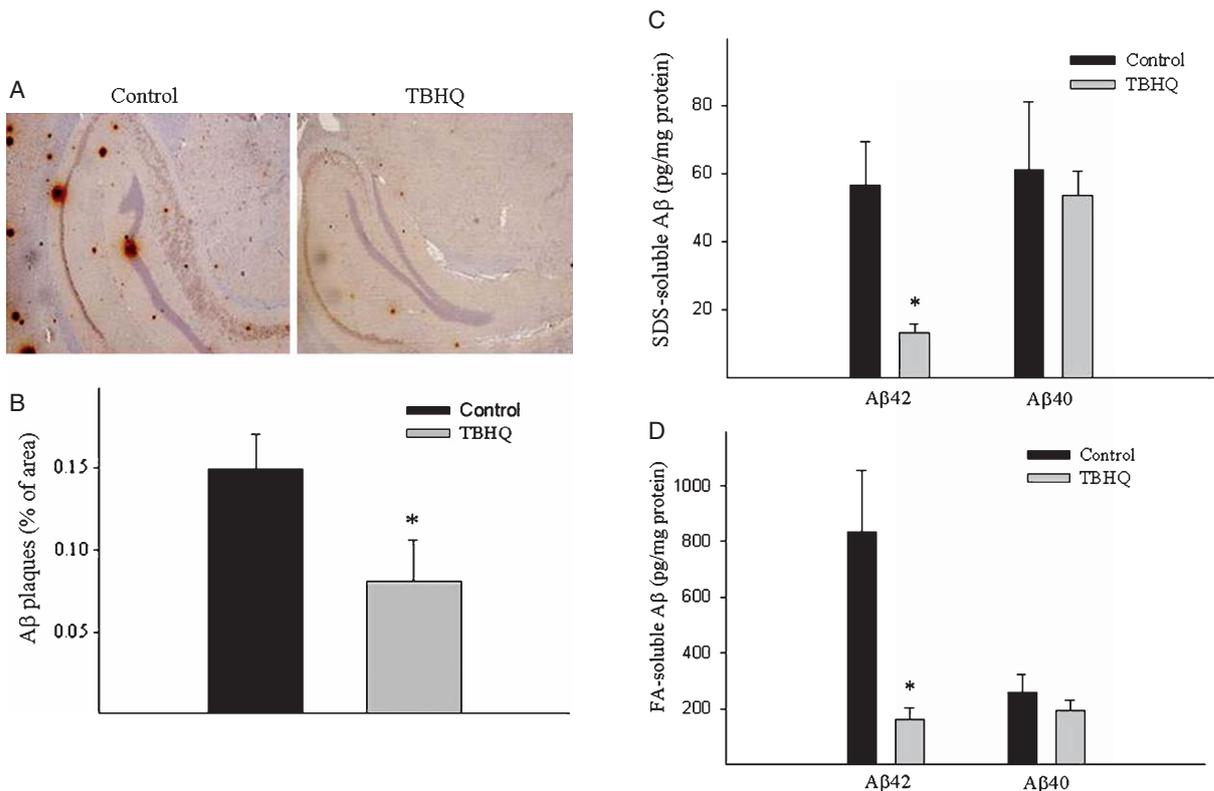


Fig. 1. Effect of TBHQ diet on A β load in the brain of A β PP/PS1 mice. Two and a half month old male A β PP/PS1 mice were fed with TBHQ or control diet for 6 weeks. A β deposits in the brain were revealed by immunohistochemical staining using antibody specific to human A β (6E10) (A) and quantified by the histomorphometry system and expressed as percentage of total area of brain (B). The amounts of A β ₄₀ and A β ₄₂, both soluble and insoluble, in the brain were determined by ELISA and calculated based on the total protein content (C&D). *Significantly different from the corresponding control diet fed mice ($p < 0.05$, $n = 5-7$).

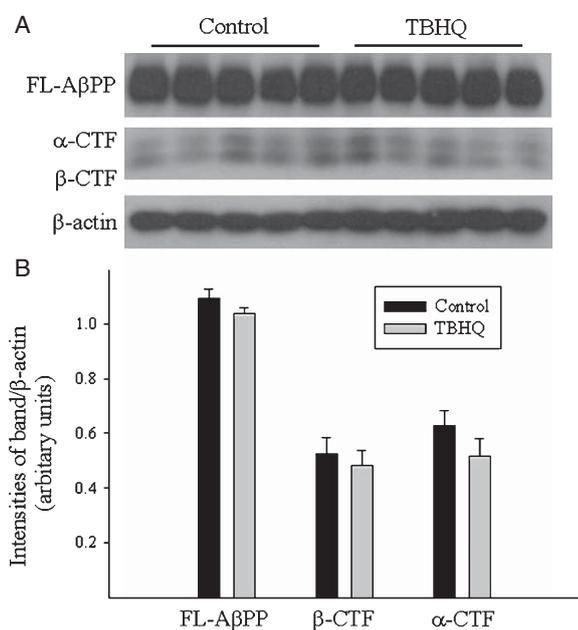


Fig. 2. Effects of TBHQ diet on the amounts of full-length A β PP, α -CTF, and β -CTF in the brain of A β PP/PS1 mice. Full-length A β PP (FL-A β PP) and α / β -CTF in the brain of A β PP/PS1 mice were determined by western analyses using 4–20% gradient SDS-PAGE and anti-human A β antibody (6E10) or C terminus of human A β PP (CT695) (top panel). The intensities of the bands were semi-quantified by densitometric scanning and normalized by β -actin (bottom panel). *Significantly different from control diet fed mice ($p < 0.05$, $n = 3-5$).

soluble and insoluble A β_{42} but had no significant effect on A β_{40} levels (Fig. 1C, D). No A β accumulation was detected in A β PP/PS1 non-transgenic mice with or without TBHQ treatment (data not shown).

Effects of TBHQ diet on the expression of full length A β PP, α -CTF, and β -CTF in the brain of A β PP/PS1 mice

To explore the mechanism whereby TBHQ reduced brain A β load in A β PP/PS1 mice, we measured the levels of full-length A β PP (FL-A β PP), α -C terminal fragments (α -CTF) and β -C terminal fragments (β -CTF) in the brain by Western blot. As shown in Fig. 2, there were no significant differences in the amounts of FL-A β PP (~100 kDa), α -CTF, or β -CTF between control diet and TBHQ diet fed mice. These results suggest that TBHQ diet reduces A β load in the brain of A β PP/PS1 mice not by altering A β PP expression/processing or inhibiting A β production but by stimulating A β degradation and/or efflux from the brain.

Effects of TBHQ diet on the expression of plasminogen activator inhibitor 1 and the activities of plasminogen activators as well as plasmin in the brain of A β PP/PS1 mice

Plasminogen activator inhibitor 1 (PAI-1) is a primary inhibitor of tissue type and urokinase type plasminogen activator (tPA and uPA), which convert plasminogen into plasmin, a serine protease that plays a critical role in A β degradation [28–32]. Our previous studies have shown that PAI-1 expression is increased in the brain of AD patients as well as in animal models of AD; knockout of the PAI-1 gene, on the other hand, reduced brain A β burden in A β PP/PS1 transgenic mice [24], suggesting a critical role of PAI-1 in brain A β accumulation. To further elucidate the mechanism whereby TBHQ reduced brain A β load in A β PP/PS1 mice, we compared PAI-1 protein levels as well as tPA, uPA, and plasmin activities between control diet and TBHQ diet fed mice. ELISA results showed that TBHQ diet significantly reduced PAI-1 protein levels in the brain of A β PP/PS1 mice (Fig. 3A). Associated with inhibition of PAI-1 expression, TBHQ diet increased the activities of tPA and uPA as well as plasmin (Fig. 3B, C and D).

Effects of TBHQ diet on the expression of low density lipoprotein receptor related protein 1 in the blood-brain barrier and the levels of A β in the plasma

LRP-1, a multi-function endocytic receptor expressed on endothelium of the blood-brain barrier (BBB), plays a major role in transporting A β out of the brain (efflux) [33–36]. It has been reported that LRP-1 expression in the brain is decreased during aging and in AD, associated with decreased A β efflux from and increased A β accumulation in the brain [25, 37]. Therefore, we examined whether TBHQ diet reduced brain A β load by increasing the expression of LRP-1 at the BBB. Immunohistochemical staining results showed that feeding mice with TBHQ diet significantly increased the abundance of LRP-1 protein at the BBB in A β PP/PS1 mice (Fig. 4A, B). Western analyses further confirmed that LRP-1 protein levels were increased in the brain of TBHQ fed A β PP/PS1 mice as compared with control diet fed A β PP/PS1 mice (Fig. 4C, D).

To further reveal whether increased LRP-1 expression was accompanied by an increase in A β efflux from the brain, we measured A β_{40} and A β_{42} levels in the plasma of A β PP/PS1 mice fed with control or TBHQ

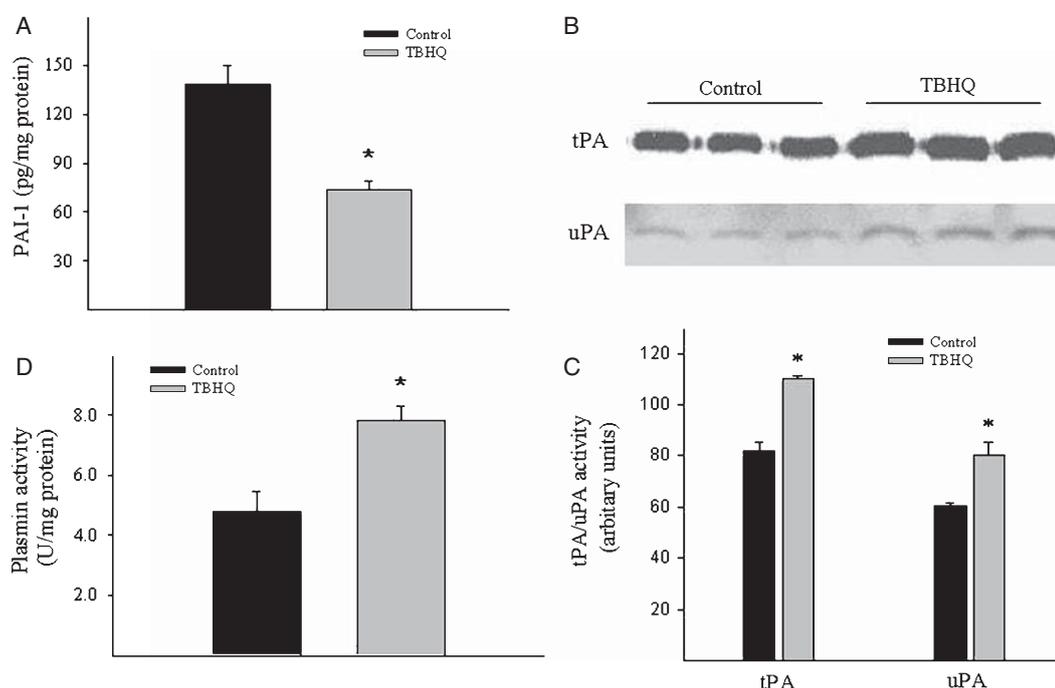


Fig. 3. Effects of TBHQ diet on PAI-1 protein expression and the activities of tPA, uPA, and plasmin in the brain of A β PP/PS1 mice. (A) PAI-1 protein levels in the brain tissue were determined by ELISA. (B) The activities of tPA and uPA in brain tissue were determined by zymography. (C) the intensities of the bands semi-quantified by densitometric scanning using Image J software. (D) Plasmin activity was determined using a specific chromogenic substrate as described in the Materials and Methods section. *Significantly different from control mice ($p < 0.05$, $n = 5-6$).

diet. The results showed that TBHQ diet increased both A β_{40} and A β_{42} levels in the plasma of A β PP/PS1 mice (Fig. 5).

Effects of TBHQ on glutathione content, NADPH oxidase2 expression, and lipid peroxidation level in the brain of A β PP/PS1 mice

Although it has been reported that TBHQ increases brain antioxidant capacity including the concentration of GSH, the most abundant intracellular free thiol and an important antioxidant, whether TBHQ can alleviate oxidative stress in AD is unknown. NADPH oxidases (Noxs) are important producers of ROS for both phagocytic and non-phagocytic cells while Nox2 is the prototype of NADPH oxidase family. To elucidate whether TBHQ diet can ameliorate oxidative stress levels in AD, we assessed the effects of TBHQ diet on the levels of Nox2 protein, GSH, and lipid peroxidation (4-HNE) in the brain of A β PP/PS1 mice. The results showed that TBHQ diet inhibited Nox2 protein expression (Fig. 6A,B), increased GSH level (Fig. 6C), and suppressed lipid peroxidation (Fig. 6D) in the brain of A β PP/PS1 mice.

DISCUSSION

AD is a devastating neurodegenerative disease that affects about 27 million people worldwide [38]. Despite extensive study, the etiology of AD is still unclear and there is no effective treatment for this devastating disease. In this study, we showed that feeding 2.5 month old male A β PP/PS1 mice with a diet containing a synthetic phenolic antioxidant TBHQ, which is widely used in food and cosmetic products as a preservative, for 6 weeks dramatically reduced their brain A β load. Further studies suggest that TBHQ reduces brain A β level probably by 1) suppressing PAI-1 expression/activity and thereby increasing the activities of tPA/uPA and plasmin and A β degradation; and 2) increasing LRP-1 expression at the BBB and thereby A β efflux from the brain. We also showed that TBHQ increased GSH content and suppressed Nox2 expression as well as lipid peroxidation in the brain of A β PP/PS1 mice. Therefore, it is speculated that TBHQ reduces brain oxidative stress levels in A β PP/PS1 mice, which leads to suppression of PAI-1 but induction of LRP-1 expression and consequently stimulation of A β degradation and efflux from the brain (Fig. 7).

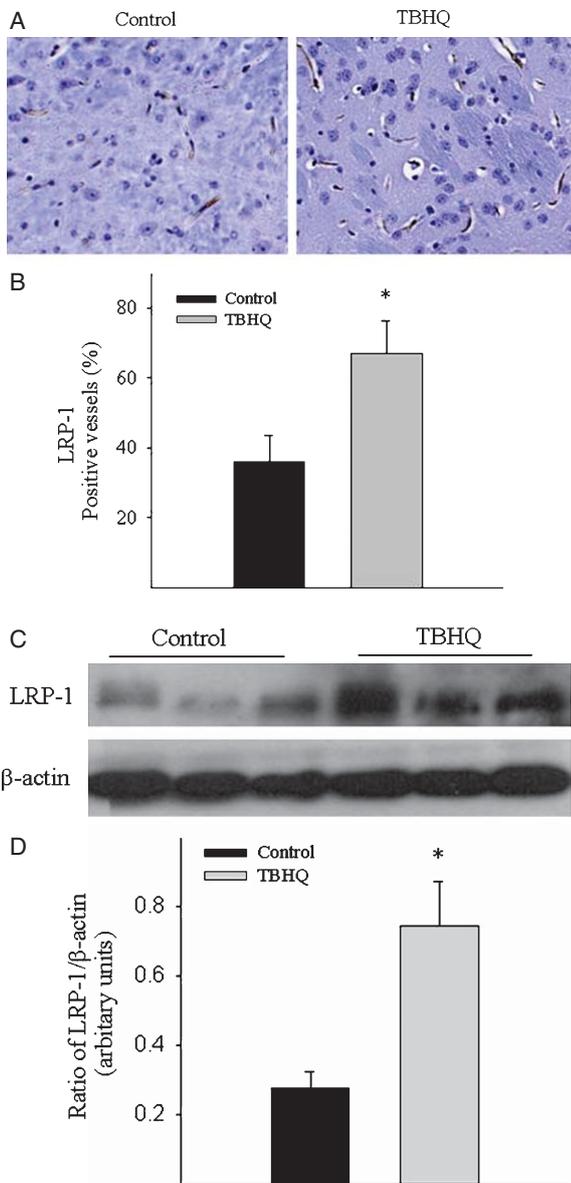


Fig. 4. Effects of TBHQ diet on the expression of LRP-1 at the BBB in A β PP/PS1 mice. A) Representative immunostaining pictures of LRP-1 protein in the brains of control-diet and TBHQ-diet fed mice. B) Quantification of LRP-1 positive blood vessels in cerebral cortex/hippocampus, expressed as percentage of total number of blood vessels in the same area of the brain. C) Representative western blotting picture of LRP-1 protein in cerebral cortex and hippocampus of A β PP/PS1 mice. D) Semi-quantitative data of western blotting. *Significantly different from control diet fed A β PP/PS1 transgenic mice ($p < 0.05$, $n = 5$).

Although it has been well documented that A β production is increased in familial AD due to mutations in the A β PP or PS1 and PS2 genes, the mechanism underlying A β accumulation in the brain of sporadic

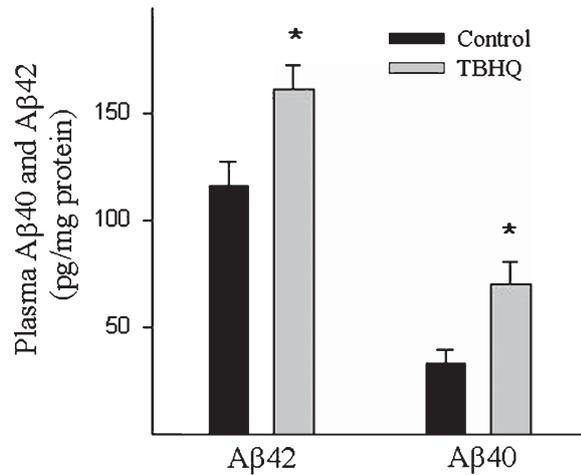


Fig. 5. Effect of TBHQ on plasma A β ₄₂ and A β ₄₀ levels in A β PP/PS1 mice. A β ₄₀ and A β ₄₂ levels in the plasma were determined by ELISA. *Significantly different from control diet fed mice ($p < 0.05$, $n = 5$).

AD is unknown. The levels of A β in the brain represent a dynamic equilibrium state as a result of their biosynthesis, degradation, influx, and efflux. Increasing evidence suggests that decrease in the clearance capacity, including degradation and efflux, may contribute importantly to A β accumulation during aging and in AD. Plasminogen activator inhibitor 1 (PAI-1) is a primary inhibitor of tissue type and urokinase type of plasminogen activators (tPA and uPA, respectively), which convert plasminogen into plasmin, a serine protease that plays a critical role in A β degradation [28–32, 39–41]. It was reported that amyloid deposition in the brain was increased in PAI-1 transgenic mice [42, 43]. Previous studies from this and other laboratories showed, on the other hand, that PAI-1 expression was increased in the brain of AD patients and in A β PP or A β PP/PS1 transgenic mice, well established animal models of AD [24, 31, 44, 45]. Knockout of the PAI-1 gene or inhibition of PAI-1 activity with PAI-1 specific inhibitors reduced brain A β burden and reversed cognitive deficits in these AD model mice [24, 25]. These data strongly suggest that increased PAI-1 expression contributes importantly to the development of amyloidosis in AD. In this study, we further showed that TBHQ administration significantly reduced brain A β load in A β PP/PS1 mice, which was associated with an inhibition of PAI-1 expression and increases in the activities of tPA, uPA, and plasmin (Fig. 3). No significant effects of TBHQ on the expression of full length A β PP or α -/ β -CTFs (Fig. 2). These data further support the notion that PAI-1 plays a pivotal role

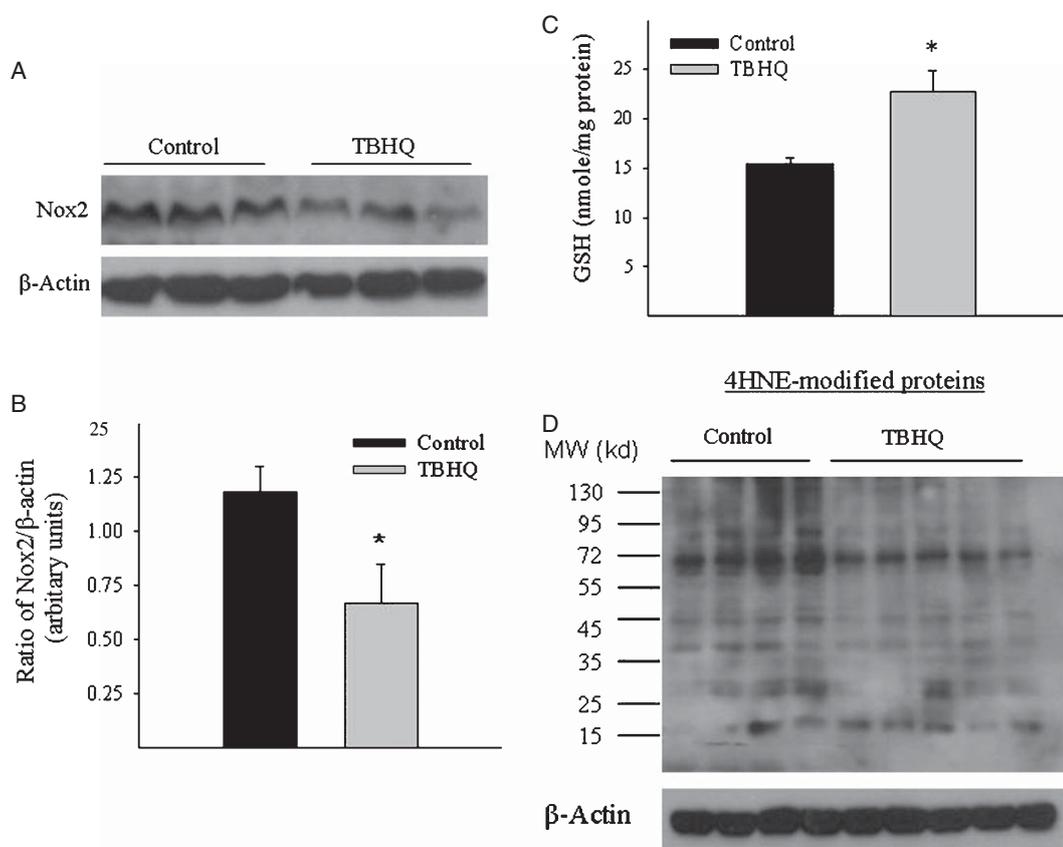


Fig. 6. Effects of TBHQ diet on brain oxidative stress and antioxidant levels in A β PP/PS1 mice. A) Representative western blotting picture of Nox2 protein in the brain of A β PP/PS1 transgenic mice. B) Semi-quantitative data of western blotting. *Significantly different from control diet fed A β PP/PS1 transgenic mice ($p < 0.05$, $n = 5-6$). C) GSH content in the brain. *Significantly different from control diet fed mice ($p < 0.05$, $n = 5-6$); D) Representative western blotting picture of 4-HNE modified proteins in the brain of A β PP/PS1 mice. β -actin was used to show equal protein loading between samples.

in AD pathology. These results also suggest that TBHQ reduces brain A β load not by inhibiting A β PP/A β synthesis but, at least in part, by inhibiting PAI-1 expression and thereby stimulating A β degradation.

The mechanism underlying the inhibition of PAI-1 expression in the brain of A β PP/PS1 mice by TBHQ is unknown at the moment. It has been well documented that oxidative stress is increased with age and in AD. It has also been demonstrated that increased oxidative stress contributes importantly to the pathogenesis of AD, including brain A β accumulation, although the underlying mechanism remains largely undefined. PAI-1 is an early response protein and can be induced by different cytokines/chemokines/growth factors including transforming growth factor beta (TGF- β), tumor necrosis factor alpha (TNF α), and angiotensin II. Interestingly, studies from this and other laboratories have shown that PAI-1 expression

is redox regulated and that ROS mediate the induction of PAI-1 by several stimuli in different types of cells [46-52]. The proximal promoter region of the PAI-1 gene contains the binding sites for the activator protein 1 (AP-1), specificity protein 1 (SP-1), and NF- κ B, three redox sensitive transcription factors. Our previous studies showed that treatment of fibroblasts with GSH, the most abundance intracellular free thiol and an important antioxidant, or GSH ester blocked the binding of AP-1 and SP-1 to the promoter of the PAI-1 gene and selectively inhibited TGF- β 1-induced PAI-1 mRNA expression [26, 46, 53]. Our previous studies further showed that, in addition to GSH, NADPH oxidase inhibitor diphenyleneiodonium and superoxide dismutase/catalase mimetic MnTBAp also dramatically reduced TGF- β -induced PAI-1 protein expression. Therefore, it was hypothesized that the redox status of cell environment regulates PAI-

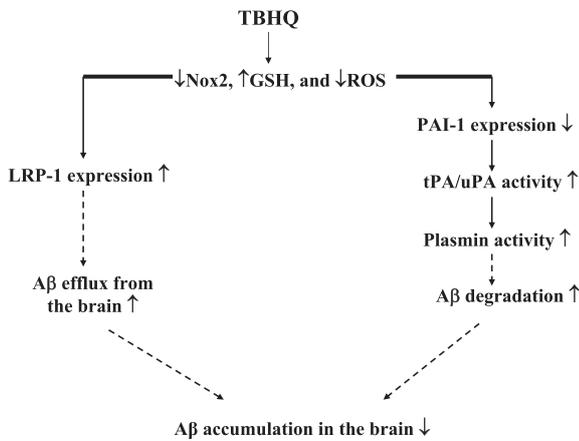


Fig. 7. The hypothetic mechanisms by which TBHQ reduces brain A β load in AD. TBHQ increases antioxidant capacity and reduces oxidative stress levels in the brain, which leads to suppression of the expression of PAI-1, a redox regulated protein, and induction of LRP-1 and thereby increased A β degradation/efflux from the brain. Solid lines represent evidence demonstrated directly from the present study.

LRP-1 expression through modulating the binding of the transcription factors AP-1 and SP-1 to the promoter of the PAI-1 gene. Importantly, previous studies from this lab have shown that GSH concentration decreases with age in several organs including the brain in wild type mice and rats as well as in the plasma of AD patients [54–58]. These data suggest that decrease in GSH level or increase in oxidative stress may underlie the increased PAI-1 expression during aging and in AD. TBHQ can directly scavenge free radicals and also induce the expression of the many enzymes involved in antioxidant defense including glutamate cysteine ligase (GCL), the rate-limiting enzyme in *de novo* GSH synthesis. In this study, we showed that TBHQ diet increased the concentration of GSH, suppressed the expression of NADPH oxidase 2 (Nox2), and reduced lipid peroxidation level in the brain of A β PP/PS1 mice. These results further confirm antioxidant property of TBHQ. These results also suggest that feeding mice with TBHQ diet suppresses PAI-1 gene expression probably by reducing oxidative stress level in the brain.

LRP1 is a multi-function endocytic receptor expressed on endothelium of the BBB, which plays a major role in transporting A β out of the brain [33–36]. It has been reported that the expression of LRP-1 at the BBB is decreased in AD, a pre-AD stage referred as amnesic mild cognitive impairment, and in well established animal model of AD, associated with a decreased A β efflux and an increased accumulation of A β in the brain [25, 37, 59–61]. LRP-

1 expression/activity in the brain is also decreased with increasing age [25, 62]. Furthermore, it has been reported that LRP-1 antisense selectively decreased LRP-1 expression, reduced BBB clearance of A β ₄₂, increased brain A β ₄₂ level, and impaired learning function in mice [63]. Together, these results suggest that decrease in the expression of LRP-1 during aging and in AD contributes importantly to the pathophysiology of AD. In this study, we showed that TBHQ feeding significantly increased the expression of LRP-1 protein at the BBB, which was accompanied by elevation of plasma A β ₄₀ and A β ₄₂ levels. These data suggest that TBHQ reduces brain A β load in A β PP/PS1 mice probably also through increasing LRP-1 expression and thereby A β efflux from the brain.

The mechanism underlying the decrease in the expression of LRP-1 in the brain during aging and in AD is unknown. Although there is no published data showing that LRP-1 gene expression is redox regulated, a recent study showed that LRP-1 protein in the hippocampus from AD patients was oxidatively modified (4-HNE modification) [64]. The authors proposed that A β induced oxidative modification of its transporter LRP1, leading to decreased A β efflux and increased A β deposition in the brain [64]. Whether increased oxidative stress is responsible for the decrease of LRP-1 expression during aging and in AD and whether TBHQ induces LRP-1 expression by reducing oxidative stress level in the brain of A β PP/PS1 mice is unknown and warrant further investigation. Understanding the mechanism regulating the expression of LRP-1, a major A β transporter from the brain to the blood, may lead to discovery of novel therapeutic agents for this devastating disease.

Overall, the present study demonstrated for the first time that TBHQ administration reduced brain A β level in A β PP/PS1 mice. The results also suggest that TBHQ does so by suppressing oxidative stress and thereby the expression/activity of PAI-1 and by stimulating the expression of LRP-1, which leads to increased degradation/clearance of A β from the brain. Together, the results suggest that TBHQ may have therapeutic potential for AD.

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Authors' disclosures available online (<http://www.j-alz.com/disclosures/view.php?id=882>).

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