

Coenzyme Q10 Attenuates β -Amyloid Pathology in the Aged Transgenic Mice with Alzheimer Presenilin 1 Mutation

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Abstract One of the neuropathological features of Alzheimer's disease (AD) is the deposition of senile plaques containing β -amyloid ($A\beta$). There is limited evidence for the treatment to arrest $A\beta$ pathology of AD. In our present study, we tested the effect of coenzyme Q10 (CoQ10), an endogenous antioxidant and a powerful free radical scavenger, on $A\beta$ in the aged transgenic mice overexpressing Alzheimer presenilin 1-L235P (leucine-to-proline mutation at codon 235, 16–17 months old). The treatment by feeding the transgenic mice with CoQ10 for 60 days ($1,200 \text{ mg kg}^{-1} \text{ day}^{-1}$) partially attenuated $A\beta$ overproduction and intracellular $A\beta$ deposit in the cortex of the transgenic mice compared with the age-matched untreated transgenic mice. Meanwhile, an increased oxidative stress reaction was detected as evidenced by elevated level of malondialdehyde (MDA) and decreased activity of superoxide dismutase (SOD) in the transgenic mice relative to the wild-type mice, and supplementation of CoQ10 partially decreased MDA level and upregulated the activity of SOD. The results indicate that oxidative stress is

enhanced in the brain of the transgenic mice, that this enhancement may further promote $A\beta_{42}$ overproduction in a vicious formation, and that CoQ10 would be beneficial for the therapy of AD.

Keywords Alzheimer's disease · Presenilin 1 · $A\beta_{42}$ · Oxidative stress · Coenzyme Q10

Abbreviations

AD	Alzheimer's disease
$A\beta$	β -amyloid
CoQ10	coenzyme Q10
PS-1	presenilin 1
MDA	malondialdehyde
SOD	superoxide dismutase

Introduction

Alzheimer's disease (AD) is the most common cause of progressive decline of cognitive function in aged humans and is characterized by the presence of numerous senile plaques containing β -amyloid ($A\beta$). It is now generally accepted that $A\beta$ plays an initial role in the pathogenesis of AD (Golde et al. 2000; Kowalska et al. 2004). Therefore, depressing $A\beta$ overproduction would be a crucial step for AD prevention or therapy.

Studies showed that mutations in presenilin 1 (PS-1) have been associated with familiar AD patients (Schellenberg et al. 2000; Xia 2000; St George-Hyslop 2000; Kovacs and Tanzi 1998). PS-1 mutation causes a selective increase in $A\beta_{42}$ relative to $A\beta_{40}$ both in cultured cells and brains of transgenic mice (Borchelt et al. 1996; Duff et al. 1996; Citron et al. 1997). Chui et al. (1999) has reported that

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transgenic mice bearing L286V PS-1 mutation exhibited significantly higher intracellular A β deposit-positive neurons than control and wild-type transgenic mice and neurodegeneration in AD without formation of extracellular plaques. More and more studies support the early pathogenic role of intracellular deposit of A β in the pathogenesis of AD (Ohyagi and Tabira 2006; Gouras et al. 2005). Thus, transgenic mice expressing mutant PS-1 could serve as an ideal early AD model to explore the pathophysiology of AD and monitor therapeutic effects of drugs. Furthermore, the effect of pathological mutation of the PS-1 gene (L235P: substitution of leucine by proline at codon 235) in AD was seldom explored.

Accumulating evidence indicates the involvement of oxidative stress in the pathogenesis of AD (Liu et al. 2007; Filipcik et al. 2006; Sultana et al. 2006). In vitro studies have demonstrated that A β can induce oxidative stress. For example, A β has been shown to increase the levels of hydrogen peroxide and lipid peroxides in cultured cells (Behl et al. 1994) and antioxidants such as vitamin E protect neurons against A β -induced cytotoxicity (Chan and Shea 2006; Cole and Frautschy 2006; Sultana et al. 2005; Dai et al. 2007; see review Butterfield et al. 1999). Furthermore, in vitro study showed that oxidative stress is correlated with A β overproduction (Paola et al. 2000). However, there is limited in vivo evidence about the relationship between A β and oxidative stress and no effective measure to arrest these pathologies.

Coenzyme Q10 (CoQ10) is an endogenous antioxidant and powerful free radical scavenger (Gazdik et al. 2003). Synthesis of CoQ10 decreases with aging (Borek 2004). Evidence has shown that CoQ10 has potentially neuroprotective effects on neurodegenerative diseases such as Parkinson's disease (Shults et al. 2002), amyotrophic lateral sclerosis (Ferrante et al. 2005), and Huntington disease (Beal et al. 2003). However, there are few reports on the effect of CoQ10 on AD.

Huang et al. (2003) has reported that the L235P PS-1 causes a selective increase in A β 42 relative to A β 40 in the brain of transgenic mice. In our present study, we tested the effect of CoQ10 on A β pathology using the transgenic mouse model.

Materials and Methods

Antibodies and Chemicals

Bicinchoninic acid (BCA) protein detection kit, chemiluminescent substrate kit, and phosphocellulose units and 3,3',5,5'-tetramethyl benzidine (TMB) were from Pierce Chemical Company (Rockford, IL). Oregon Green 488-conjugated goat anti-rabbit IgG (H+L) were from Molecular Probes (Eugene,

OR). The assay kits of malondialdehyde (MDA) and superoxide dismutase (SOD) were purchased from Jiancheng Institute Biotechnology (Nanjing, China). The Congo Red kit and synthetic mouse A β 1-40 and A β 1-42 were from Sigma.

Animals

All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory animals (NIH Publication no. 8023, revised 1978), and all efforts were made to minimize animal suffering and to reduce the number of animals used. Sixteen- to seventeen-month-old female animals were divided into three groups: untreated wild type ($n=11$), untreated transgenic mice comprising mutant L235P PS-1 ($n=10$); Treated transgenic mice ($n=10$), which were fed with CoQ10 (1,200 mg kg⁻¹ day⁻¹, Tishcon) for 60 days. The dosage of CoQ10 was referenced to a previous report (Li et al. 2007). The mice were derived from the second back-cross of PS-1 transgenic mice to F1 of C57BL/6J \times A2G. The animals were caged singly in a temperature- (22°C) and humidity- (25%) controlled animal room, under a 12:12-h light-dark cycle (lights on from 07:00 to 19:00 hours) and kept under ad libitum food and water throughout.

Genotyping

The genotypes of the mice were determined using a standard protocol for polymerase chain reaction.

Determination of A β 40 and A β 42 by Sandwich Enzyme-linked Immunosorbent Assay

At the end of treatment, the animals were killed, and their brains were dissected on ice. The cerebral cortex was separated and stored at -70°C until use.

Levels of mouse A β 40 and A β 42 were quantified by sandwich enzyme-linked immunosorbent assay as we previously described (Qu et al. 2005; Wang et al. 2006). Briefly, the cortex was separately homogenized in 1 ml of 70% formic acid. The supernatant was collected after centrifugation of the homogenate at 10,000 \times g for 1 h. Ninety-six-well plates (Biomat, Rovereto TN, Italy) were coated with G2-10 specific for A β 40 and G2-11 specific for A β 42, as capture antibodies. The presence of A β 40 and A β 42 was then detected specifically by antibody biotin-WO2 (A β eta, Germany) and further developed with horse radish peroxidase-NeutrAvidin (A β eta) by using TMB as the substrate. Values were normalized with the standard curve generated using synthetic A β 1-40 and A β 1-42.

Immunofluorescence and Congo Red Staining

For immunohistochemical studies, mice were deeply anesthetized and transcardially perfused with 100 ml 0.01 M phosphate-buffered saline, pH 7.4, first and then 100 ml 4% paraformaldehyde solution. The brain was dissected out and was postfixed in the same 4% paraformaldehyde solution for 3–4 h and then placed in phosphate-buffered 30% sucrose overnight. On the following day, 30- μ m frozen sections were coronally cut on a sliding microtome (AO Scientific Instruments, USA). Immunofluorescence labeling was performed following the procedure. Briefly, free-floating slices were incubated at 4°C overnight with anti-A β 42 (1:1,000, Biosource, Camarillo, CA). The immunoreactivity of anti-A β 42 was probed using Oregon Green 488-conjugated goat anti-rabbit IgG (H+L). The adjacent coronal sections of mouse brains were also stained with Congo Red according to the instruction provided by the kit. The images were taken using a laser confocal microscope (Bio-Rad, Hertfordshire, UK). A β 42- and Congo Red-positive cells were counted using a stereological system (Stereo Investigator 2000 4. 3, MicroBrightfield, USA) in eight microscopic fields per animal under \times 400 magnification ($n=4$ in each group).

Assay of MDA Content and SOD Activity

For the measurement of the product of lipid peroxidation and SOD activity, mouse cortex was homogenized in ice-cold 20 mM Tris-HCl buffer (pH 7.4). Lipid peroxidation product was determined by measuring the MDA formed by the thiobarbituric acid reaction. The contents of lipid peroxidation product MDA in the cortex of the mice were measured with the thiobarbituric acid reaction to indicate the lipid peroxidation according to the instructions provided by the kit. The assay for total SOD was based on its ability to inhibit the oxidation of oxyamine by the xanthine-xanthine oxidase system. The red product (nitrite) produced by the oxidation of oxyamine had an absorbance at 550 nm.

One unit of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%. The concentration of protein in the cortex extract was estimated using the BCA kit according to manufacturer's instruction.

Statistical Analysis

Data was expressed as mean \pm SD and analyzed using SPSS 13.0 statistical software (SPSS, Chicago, IL). The one-way analysis of variance procedure followed by least significant difference post-hoc tests was used to determine the different means among groups. The level of significance was set at $P<0.05$.

Results

Effects on CoQ10 in A β 42 Levels and Intracellular A β 42 Deposits

The expression of the human L235P PS-1 caused an obvious enhancement in the production of A β 42 in the cortex of the transgenic mice, relative to the wild type mice (4.87 ± 0.65 vs 1.43 ± 0.38 , $P<0.01$, Fig. 1a). To investigate the effects of CoQ10 on A β production, the transgenic mice were fed with CoQ10 with a dosage of $1,200$ mg kg^{-1} day^{-1} . It was shown that A β 42 in the treatment group with CoQ10 was significantly decreased by about 23% in the cortex of the transgenic mice, relative to the age-matched untreated transgenic mice (3.74 ± 0.46 vs 4.87 ± 0.65 , $P<0.01$, Fig. 1a). No significant difference of A β 40 levels was observed among all groups of mice (Fig. 1b).

In addition, we also observed by immunofluorescence labeling an increased staining using specific antibody against A β 42 in some cortical neuronal cells in the transgenic mice, but very few intracellular A β 42-positive cells were seen in the wild-type mice (Fig. 2a). The quantitative analysis revealed that there were significantly more neuronal cells with intracellular A β 42 deposit in the

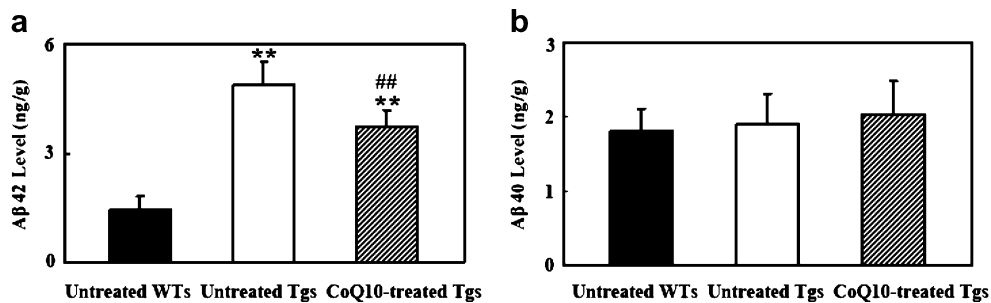


Figure 1 Effects of CoQ10 on levels of A β 40 and A β 42 in the cortex of the transgenic mice. A β 42 level was significantly increased in the cortex of the transgenic mice compared with the wild-type mice (a, $P<0.01$). A β 42 level was significantly attenuated by the treatment

of CoQ10 (a, $P<0.01$). No significant difference of A β 40 level was detected (b). Data were mean \pm SD ($n=5$). Double asterisk, $P<0.01$ vs untreated WTs, double number sign, $P<0.01$ vs untreated Tgs

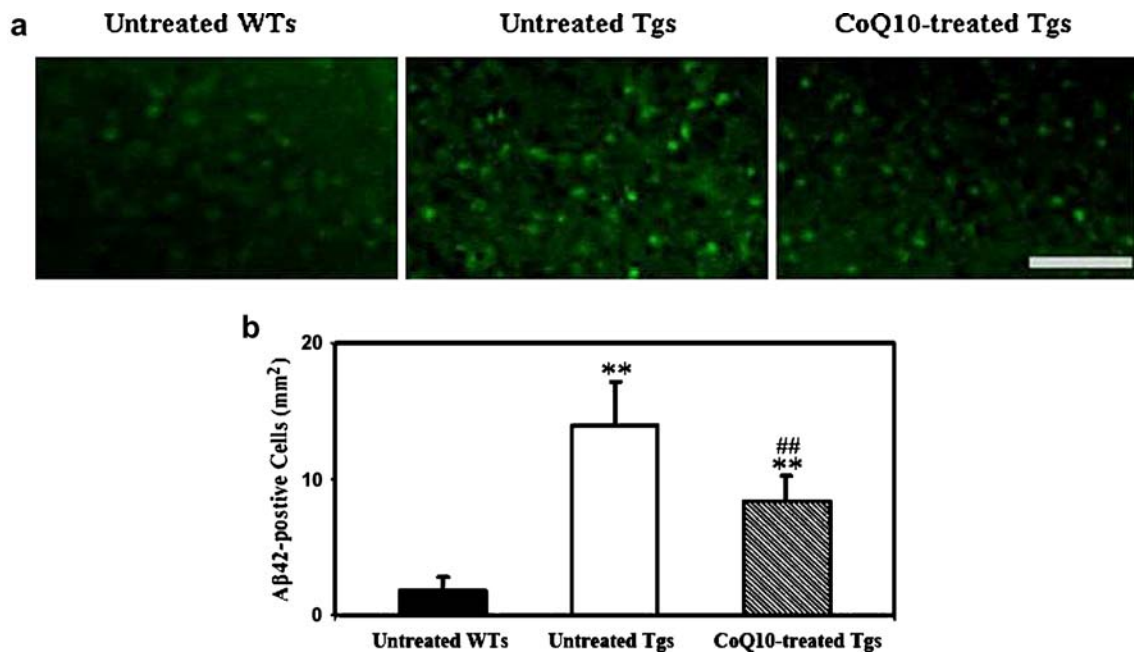


Figure 2 Effects of CoQ10 on immunoreactivity of Aβ42 in the cortex of the transgenic mice. The brain slices were immunostained with anti-Aβ42. Intracellular deposit of Aβ42 was seen in the aged transgenic mice (a). Quantification of intracellular Aβ42-positive cells

(b). Data were mean±SD (n=4). Double asterisk, $P<0.01$ vs untreated WTs, double number sign, $P<0.01$ vs untreated Tgs. Scale bar=50 μm

untreated transgenic mice than in the wild-type mice (Fig. 2b). The intracellular Aβ42-positive cells in the transgenic mice were significantly decreased after the treatment of CoQ10 (Fig. 2b). Similar results were observed in hippocampus (data not shown). In addition, to further confirm the results from Aβ42 staining, the adjacent brain sections were stained with Congo Red, a specific amyloid dye, and similar results were observed (Fig 3a,b). However, we failed to observe extracellular Aβ deposits with the

Aβ40 and Aβ42 antibodies and Thioflavin S and Congo Red staining in the animals from all groups (data not shown).

Effects of CoQ10 on MDA Content and SOD Activity in the Transgenic Mice

To investigate the change of oxidative stress in the transgenic mice and to test the effect of CoQ10, we measured the level of MDA and the activity of SOD, two

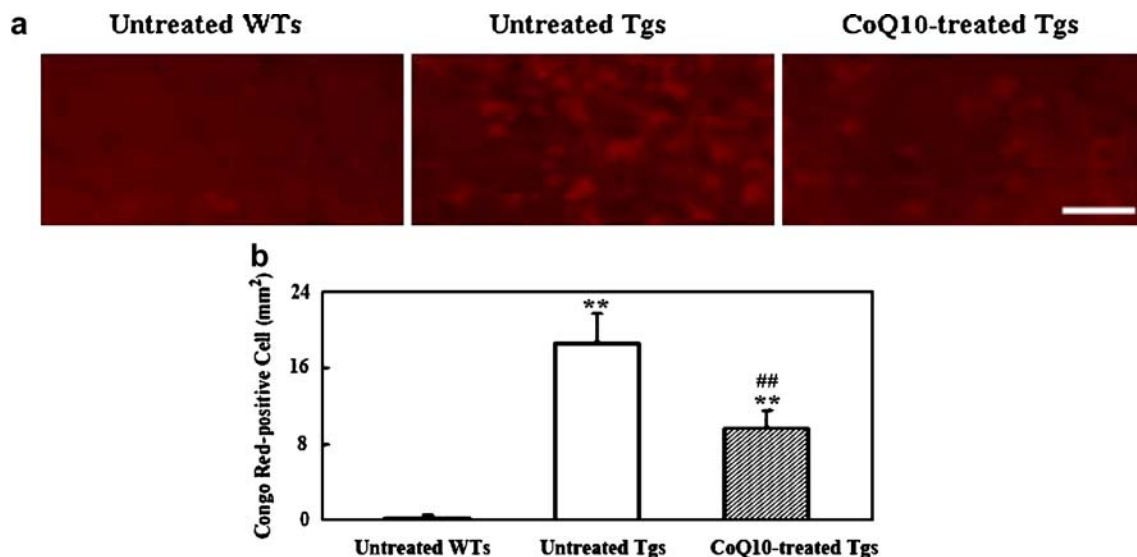


Figure 3 Effects of CoQ10 on Congo Red staining in the cortex of the transgenic mice. The brain slices were stained with Congo Red. Congo Red-positive staining was seen in the aged transgenic mice (a).

Quantification of Congo Red-positive cells (b). Data were mean±SD (n=4). Double asterisk, $P<0.01$ vs untreated WTs, double number sign, $P<0.01$ vs untreated Tgs. Scale bar=20 μm

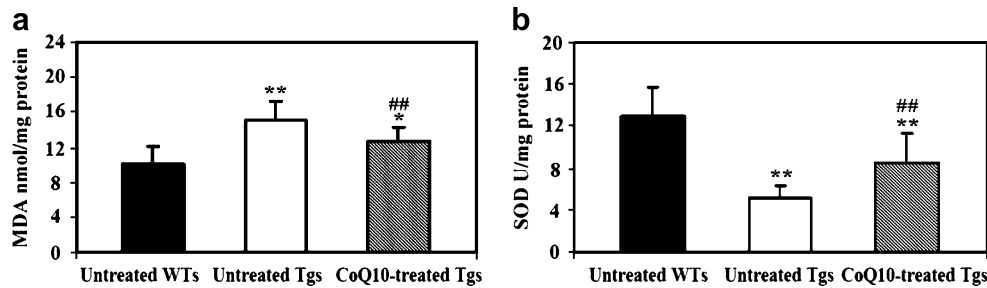


Figure 4 Effects of CoQ10 on MDA content and SOD activity in the cortex of the transgenic mice. The level of MDA in the cortex of the transgenic mice was remarkably higher than the wild-type mice (**a**, $P < 0.01$), and supplementation of CoQ10 relieved the elevation (**a**, $P < 0.05$). The activity of SOD was significantly decreased in the cortex of

the transgenic mice relative to the age-matched wild-type mice (**b**, $P < 0.01$), and supplementation of CoQ10 partially restored the activity of SOD (**b**, $P < 0.01$). Data were mean ± SD ($n = 6$). Asterisk, $P < 0.05$, double asterisk, $P < 0.01$ vs untreated WT, double number sign, $P < 0.01$ vs untreated Tgs

markers of oxidative stress, in the cortex homogenates and found that the level of MDA was significantly increased (14.98 ± 2.21 vs 10.09 ± 1.95 , $P < 0.01$, Fig. 4a) with a concomitant decreased SOD activity (5.1 ± 1.2 vs 12.9 ± 2.8 , $P < 0.01$, Fig. 4b) compared with the age-matched wild-type mice, and supplementation of CoQ10 partially arrested MDA overproduction and restored SOD activity (12.65 ± 1.72 vs 14.98 ± 2.21 , $P < 0.05$, Fig. 4a; 8.6 ± 2.7 vs 5.1 ± 1.2 , $P < 0.01$, Fig. 4b).

Discussion

AD can be initiated by A β . To find effective drugs to attenuate or stop A β overproduction has been a main topic for AD therapy. The pathological mechanism of AD through presenilin mutations is still unclear. In our present study, we found enhanced oxidative stress reaction as well as a significant increase in A β 42 and intracellular A β 42 accumulation in the cortex of the aged L235P PS-1 transgenic mice. Supplementation of CoQ10 efficiently both relieved oxidative stress and reduced A β 42 level.

Huang et al. (2003) and Chui et al. (1999) have reported a selective increase in A β 42 level and increased intracellular A β 42 deposit in the brain of transgenic mice with Alzheimer PS-1 mutation. Our current results support the previous findings.

Increasing evidence supports the involvement of oxidative stress in AD pathology (Markesbery and Carney 1999; Markesbery 1997; Durany et al. 1999). Much in vitro studies have shown that A β induced oxidative stress (Jang et al. 2007; Cetin and Dincer 2007; Boyd-Kimball and Sultana 2004). Our data that significant increased oxidative stress was detected as evidenced by increased MDA content and decreased SOD activity further support the in vitro studies. Additionally, there is much evidence showing that oxidative stress can induce A β overproduction. For

example, oxidative stress elevates beta-secretase proteins and activity and A β level in vivo in the rat retina (Xiong et al. 2007) and triggers the amyloidogenic pathway in human vascular smooth muscle cells (Coma et al. 2007). Moreover, oxidative stress induces intracellular accumulation of A β in human neuroblastoma cells (Misonou et al. 2000) and intralysosomal accumulation of Alzheimer A β in cultured neuroblastoma cells (Zheng et al. 2006). These data imply that A β not only can induce oxidative stress but its generation is also increased as a consequence of oxidative stress, thus forming a vicious circle. Thus, the enhanced oxidative stress could in return promote A β 42 overproduction observed in our present study.

CoQ10 is a powerful antioxidant that buffers the potential adverse consequences of free radicals produced during oxidative phosphorylation in the mitochondrial membrane. Recently, we showed that CoQ10 protects SHSY5Y neuronal cells from A β toxicity and oxygen-glucose deprivation by inhibiting the opening of the mitochondrial permeability transition pore (Li et al. 2005). Our present study demonstrated that CoQ10 exerted neuroprotective effects on the transgenic mice by reduction of the A β 42 level and suppression of oxidative stress. The reduction in A β 42 level could be a consequence of the attenuation of oxidative stress by CoQ10 administration. The data indicate that oxidative stress may mediate the pathogenic action of PS-1 mutation and that CoQ10 could be useful as a potential preventive or therapeutic agent for AD.

In summary, we have found in the present study (1) that the aged L235P PS-1 transgenic mice showed increased intracellular A β 42 deposit and oxidative stress as well as A β 42 overproduction and (2) that the exogenous administration of CoQ10 effectively decreased A β 42 production and intracellular accumulation and depressed oxidative stress. This is the first in vivo report showing the effect of CoQ10 on A β in AD. Our data suggest that CoQ10 would be potentially a useful drug for therapy of AD.

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