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Diet supplement CoQ_{10} delays brain atrophy in aged transgenic mice with mutations in the amyloid precursor protein: An *in vivo* volume MRI study

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Abstract. We tested the hypotheses that supplemental intake of the diet supplement Coenzyme Q_{10} (CoQ_{10}) could delay brain atrophy in double transgenic amyloid precursor protein (APP) / presenilin 1 (PS1), single transgenic APP and PS1 as well as wild type mice by volume MR image *in vivo*. One hundred and twelve mice (28 APP/PS1, 28 APP, 28 PS1 and 28 wild types) were studied. Half of each genotype group ($n = 14$ per group) was treated with CoQ_{10} 2400 mg/kg/day, and the other half with placebo for 60 days. Magnetic resonance (MR) images were used to obtain the volumes of the hemispheres and hippocampi. APP / PS1, APP, PS1 and wild type mice treated with CoQ_{10} exhibited significantly less atrophy in hemisphere and hippocampus than those receiving placebo. The neuro-protective effect of the CoQ_{10} on hemispheric volume, and hippocampal volume was related to genotype; greater in APP/PS1 than APP and PS1 mice and less in wild type mice. Our result indicated that CoQ_{10} may have therapeutic potential in the prevention and treatment of MCI and AD.

Keywords: APP/PS1 double transgenic mice, antioxidant, Alzheimer's disease, hippocampal volume, hemispheric volume

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the deposition of amyloid protein in areas of the brain that are important for memory and cognition [2,26]. This deposition is thought to be an important component of the pathology of AD [9,10]. The AD brain is under significant oxidative stress [11], and $A\beta_{1-42}$ peptide is known to cause oxidative stress *in vitro* and *in vivo* [7–9, 38]. Due to the aging boomer population, AD is beginning to approach epidemic proportions in the industrialized world. It is estimated that by the year 2025, there will be approximately 22 million cases of AD worldwide [40]. Such a large number of AD patients will have the potential to cripple the health care system. These onerous predictions underscore the necessity for the development of effective therapies

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for AD. While there is still no cure, studies have shown that early diagnosis and treatment can help to slow or alleviate the symptoms of AD [16,29,43].

In the last few years, there has been an increasing interest in the possible role of oxidative stress in AD [11,52,53]. Several reports suggested increased lipid peroxidation in the temporal cortex, increased protein oxidation in the frontal cortex, and increased DNA oxidation in the parietal cortex of AD patients [5]. Antioxidants are believed to be important in health maintenance through the modulation of oxidative processes in the body [42]. Oxidative damage with the unregulated production of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals has been implicated in a growing number of clinical disorders such as atherosclerosis [30,51], stroke [41,57], Parkinson's disease [47,50] and AD [31,32]. Mechanisms responsible for the reactive oxygen species (ROS)-mediated injury to cells and tissues mainly include lipid peroxidation, oxidative DNA damage, and protein oxidation [23], but there is also evidence that ROS can induce processes leading to cell death [12]. Indeed, unbalance in the endogenous antioxidant system can modulate cellular proliferation, either in a positive or a negative way, respectively leading to a stimulation in cell proliferation at low levels of peroxides or to apoptotic/necrotic cell death at higher concentrations [25]. Based on this background, it is clear that investigating compounds able to counteract this oxidative damage may have a relevant clinical impact for MCI and AD.

CoQ_{10} (ubiquinone) is composed of a quinine ring and a 10 isoprene unit tail and is distributed in all membranes throughout the cell. It is an electron acceptor for complexes I and II of mitochondrial electron transport chain and also serves as a powerful antioxidant [18,36,49]. CoQ_{10}'s dual functions as an integral member of the mitochondrial electron transport chain [20] and as an antioxidant make it potentially important in the treatment of AD. Evidence has accumulated that both mitochondrial dysfunction and neuronal damage due to oxidative free radicals occur in AD [5,13]. Schapira et al. reported that electron transport chain defects in AD [44]. CoQ_{10} boosts energy and enhances the immune system. CoQ_{10} may help prevent or treat AD by mediating electron transport chain defects. CoQ_{10} boosts energy and enhances the immune system. It has been tested in high dosage (4800mg/day) and showed no side effects [36]. CoQ_{10} may help prevent or treat AD [15,31,32].

The present study investigated a therapeutic CoQ_{10} believed to be candidates with strong potential to counteract oxidative damage, and therefore have a relevant clinical impact on AD and MCI.

2. Materials and methods

2.1. Transgenic mice

All experiments were conducted according to the institutional guidelines with the protocol approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong. Transgenic mice with a mixed background (C3H/HeJ/C57BL/A2G) coexpressing mutant human PS1 - Leu235Pro and APP - sew mice were crossed to generate four strains of transgenic mice: APP/PS1, APP, PS1 and wild-type. One hundred and twelve mice (28 APP/PS1, 28 APP, 28 PS1 and 28 wild types) were studied. Half of each genotype group ($n = 14$ per group) was treated with the CoQ_{10}. The mean age was 529.71 $\pm$ 40.48 days before the treatment. There were no statistical significant age differences among the four kinds of mice, also no age difference between males (524.06 $\pm$ 49.61 days) and females (535.25 $\pm$ 28.28 days) ($F = 0.098$, $P = 0.754$). The body weight of males (35.67 $\pm$ 1.34 g) was greater than that of females (27.12 $\pm$ 2.08 g) ($T = 29.33$, $P <= 0.001$) (Independent Samples test), but there were no statistically significant differences in the body weight between APP/PS1 and APP; PS1 and

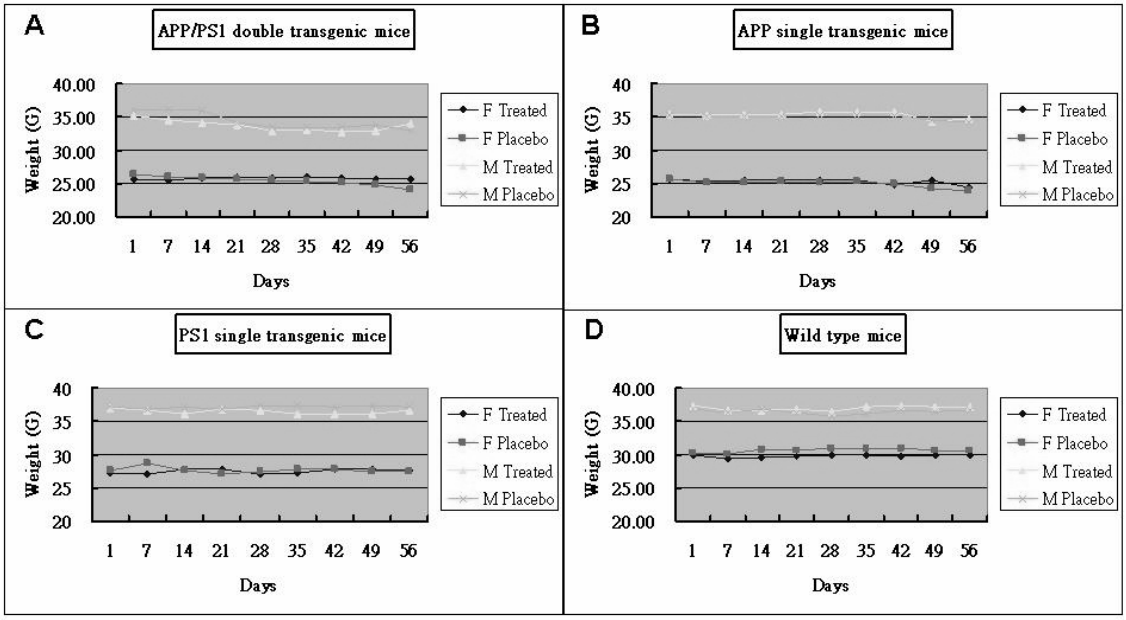

Fig. 1. Body weight of APP/PS1 double transgenic mice (A); APP (B) and PS1 (C) single transgenic mice as well as wild type mice (D).

wild-type in the same sex (multiple comparisons, Tukey HDS, $P < 0.005$). The body weight of PS1 and wild-type was greater than that of APP/PS1 and APP in the same sex (multiple comparisons, Tukey HDS, $P > 0.001$) (Fig. 1). After 60 days treatment, 4 mice in APP/PS1 groups (2 in treated group and 2 in placebo group) and 2 mice in APP groups (1 in treated group and 1 in placebo group) died.

2.2. Genotyping

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

2.3. Measurement of tissue Co_{10}

For sample preparations, 100 mg tail tissue sample was added to 500 μl distilled water and stirred for 10 minutes and then 30 μl perchloric acid (0.7 M) were added and stirred for another 10 minutes. Adjusted PH to 7.5 with approx 15 μl K2CO3 (1.75 M). Mixed and filtered it. Put it into the centrifuge at 8,000g (10,000 rpm) for 5 minutes. 100 μl supernatant was used for assay.

High-Performance Liquid Chromatography (ESA, Chelmsford, MA, USA) was used to test the tissue levels of CoQ_{10} (ng/mg) before and after the treatment (Fig. 2).

2.4. Treatment

In each of the four genotype groups ($n = 28$ each), half ($n = 14$) of the mice (the placebo group) were fed standard pelleted mouse chow. In the placebo groups, mice were given the same dosage of pure vegetable oil as that of the CoQ_{10} (Purina Test Diets, Richmond, IN) via gavage for 60 days. The treated mice ($n = 14$ per genotype) were given CoQ10 (2400 mg/kg/day, Tishcon/Gel-Tec) via gavage for 60

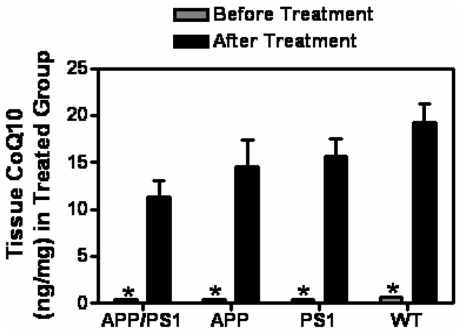

Fig. 2. Tissue CoQ10 levels (ng/mg) in treated groups. *comparison with after treatment, $P < 0.001$.

days. The amount of food intake per mouse was found to be 5–6 gm/d, with no significant difference between treated and untreated mice. During treatment, the food consumed per gram of mouse weight was stable.

2.5. MRI evaluation

MRI was performed twice on a Philips Achiva 3 T whole body MRI at the Jockey Club MRI Centre, Hong Kong with a transmit/receive wrist coil before and after treatment. To optimize contrast between grey and white matter for morphometric measures, we used a high-resolution proton density weighted (PDW) 3-dimensional (3D) turbo spin echo (TSE) sequence. Transverse brain images were taken with repetition time (TR) = 200 ms, echo time (TE) = 26 ms, flip angle = 90 degrees, water fat shift (WFS)/bandwidth (BW) = 3.029 pix/143 Hz, field-of-view (FOV) = 10 × 10 cm, matrix size = 400 × 400 producing an in-plane voxel size of 0.25 mm × 0.25 mm, TSE factor = 3, the thickness of the slices in the through-plane direction = 0.5 mm without slice gap, and number of signal averages (NSA) = 12. The hemispheric and hippocampal volumes were delineated with manual region of interest (ROI) tracing using the image processing software Analyze 6.0 (AnalyzeDirect USA). For the calculation of hemispheric and hippocampal volume, ROIs were manually drawn on whole hemisphere (HM) and whole hippocampus (H) of the PDW-3D-TSE images using Analyze (Fig. 3A). In order to correct for normal size differences between animals, volume results were expressed as a ratio relative to the intracranial volume. The intracranial volume was determined by tracing the margins of the skull's inner table (Fig. 3B). This normalizes hippocampal and hemispheric volume for inter animal differences in brain size.

2.6. Statistical analysis

Numerical results are presented as mean ± standard error (SE). Statistical comparisons of the CoQ_{10} levels in tissue, H and HM data before and after treatment were compared by Tukey Multiple Comparison in a one-way ANOVA test and two sample of t-test using SPSS 13.0 (SPSS Inc. Chicago, USA), respectively. The level of significance was set at $P < 0.05$.

3. Results

Six of the initial mice in the first experiment died before completion of the study.

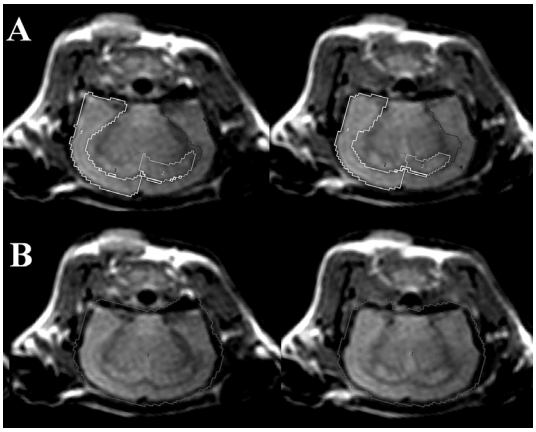

Fig. 3. PDW-3D-TSE images show the examples of drawing hemispheric, hippocampal and intracranial volumes.

3.1. Tissue CoQ_{10} (ng/mg)

Values of relative tissue CoQ_{10} levels were significantly increased after 60 days of treatment in four kinds of mice ($P < 0.001$) (Fig. 2). For the values of relative tissue CoQ_{10} levels in placebo groups, there were no significant difference between the baseline and after 60 days CoQ_{10} treatment in four kinds of mice ($P > 0.05$). Values of relative tissue CoQ_{10} levels in double transgenic mice were significantly lower than those in wild type mice ($P < 0.001$). We don't know the reason yet why tissue CoQ_{10} levels were lower in double transgenic mice. The further study should be carried out to find out the reason.

3.2. Effect of CoQ_{10} on relative hippocampus volume in APP/PS1, APP, PS1 and wild type mice

Normalized hippocampal volume was calculated by dividing hippocampal volumes (Fig. 3A) by the volume of the intracranial volume (Fig. 3B). In the placebo groups, this ratio was significantly decreased in both APP/PS1 ($n = 13$, $P < 0.005$) and APP ($n = 13$, $P < 0.01$) transgenic mice after 60 days placebo treatment (Fig. 4A, Table 1). This indicates brain atrophy in aged transgenic mouse strains that deposit amyloid (PS1 mice do not). However, there was no significant difference before and after placebo treatment in PS1 transgenic mice ($n = 14$, $P > 0.05$) or wild-type mice ($n = 14$, $P > 0.05$). In the treated groups, this ratio was not significantly changed in APP/PS1 ($n = 13$, $P > 0.05$), APP ($n = 13$, $P > 0.05$), PS1 ($n = 14$, $P > 0.05$) transgenic mice and wild-type ($n = 14$, $P > 0.05$) mice (Fig. 4B, Table 1). This indicates a neuroprotective effect of the CoQ_{10} on the volume of the hippocampus in both APP/PS1 double transgenic mice and APP single transgenic mice. The normalized hippocampal volume in APP/PS1 double transgenic mice ($n = 26$) was significantly smaller than that in wild-type mice ($n = 28$) ($P < 0.001$).

3.3. Effect of CoQ_{10} on relative hemisphere volume in APP/PS1, APP, PS1 and wild type mice

Values for the volume of the hemisphere (Fig. 3A) divided by the volume of the intracranial volume (Fig. 3B) showed significant decrease in placebo treated group among the APP/PS1 double transgenic mice ($n = 13$, $P < 0.001$), APP single transgenic mice ($n = 13$, $P < 0.005$) and PS1 single transgenic mice ($n = 14$, $P < 0.05$) after 60 days placebo treatment (Fig. 5A and Table 1) indicating the hemispheric atrophy in aged transgenic mice. However, numerical values for the volume of hemisphere showed no

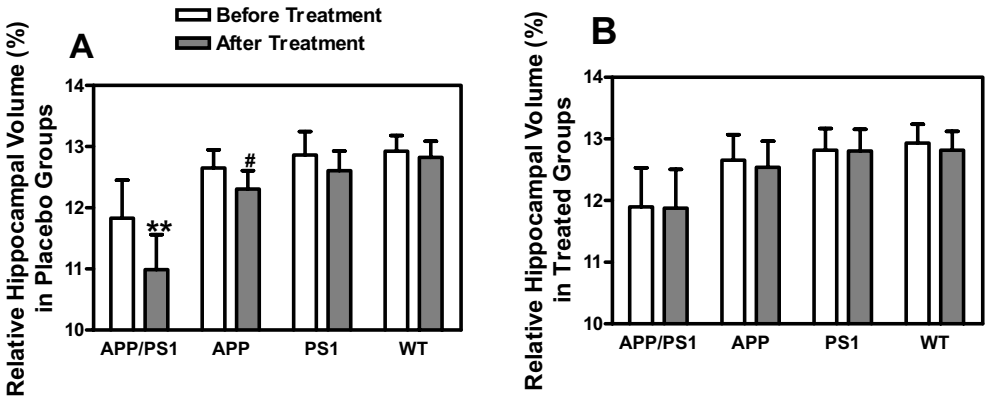

Fig. 4. Effect of 60 days treatment with the CoQ10 on relative hippocampal volume (H) in APP/PS1 ($n = 12$ in each group), APP ($n = 13$ in each group), PS1 and wild-type mice ($n = 14$ in each group). Data are expressed as a ratio relative to the intracranial volume with eight pair-wise comparisons: before the CoQ10 treatment vs. after the CoQ10 treatment in APP/PS1; APP; PS1 and in wild-type in placebo (A) and treated (B) groups. **comparison with before treated, $P < 0.005$; #comparison with before treated, $P < 0.01$. Error bars indicate standard error (SE).

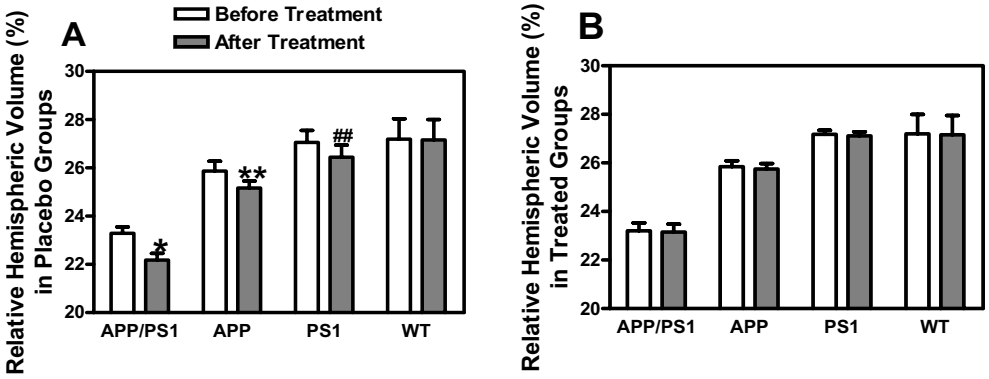

Fig. 5. Effect of 60 days treatment with the CoQ10 on relative hemispheric volume (HM) in APP/PS1, APP, PS1 and wild-type mice. Data are expressed as a ratio relative to the intracranial volume with eight pair-wise comparisons: before the CoQ10 treatment vs. after the CoQ10 treatment in APP/PS1; APP; PS1 and in wild-type in placebo (A) and treated (B) groups. *Comparison with before treated, $P < 0.001$; **comparison with before treated, $P < 0.005$; ##comparison with before treated, $P < 0.05$. Error bars indicate standard error (SE).

significant differences in treated mice before and after CoQ_{10} treatment among the four different genotype groups (Fig. 5B) ($P > 0.05$) indicating a neuroprotective effect of the CoQ_{10}. There was significant difference in the ratio of hemispheric volume between APP/PS1 double transgenic mice ($n = 26$) and wild-type mice ($n = 28$) ($P < 0.001$).

4. Discussion

Antioxidants are known to have a neuroprotective effect on a growing number of clinical disorders such as atherosclerosis [30,51], stroke [41,57], Parkinson's disease [47,50] and AD [21,31,32]. CoQ_{10} is known to have a neuroprotective effect in Parkinson's disease [46–50] and Huntington's disease [19]. Nevertheless, no studies have investigated antioxidant CoQ_{10}'s effects on mild cognitive impairment

Table 1
Mean of relative hippocampal and hemispheric volumes (%)

	APP/PS1			APP			PS1			WT		
	Before	After	P	Before	After	P	Before	After	P	Before	After	P
HV Treated	11.90	11.87	> 0.05	12.66	12.54	> 0.05	12.82	12.80	> 0.05	12.93	12.82	> 0.05
HV Placebo	11.83	10.99	< 0.005	12.65	12.31	< 0.01	12.86	12.61	> 0.05	12.93	12.82	> 0.05
HMV Treated	23.20	23.15	> 0.05	25.84	25.74	> 0.05	27.18	27.11	> 0.05	27.20	27.16	> 0.05
HMV Placebo	23.29	22.18	< 0.001	25.87	25.17	< 0.005	27.05	26.44	< 0.05	27.20	27.16	> 0.05

HV: Hippocampal volume; HMV: Hemispheric volume.

(MCI) and AD. In the present study, we found that: (1) 60 days of the diet supplement CoQ_{10} treatment preserved hippocampal volumes in APP/PS1 and APP transgenic mice, but had no effect on hippocampal volumes in PS1 transgenic mice and wild-type mice; (2) 60 days of the diet supplement CoQ_{10} treatment preserved hemispheric volume in APP/PS1, APP, and PS1 transgenic mice, but not in wild-type mice.

No hippocampal atrophy in APP/PS1 double transgenic mice and APP single transgenic mice; and hemispheric atrophy in 3 transgenic strains were observed in the diet supplement CoQ_{10} treated animals. The neuroprotective effect of the diet supplement CoQ_{10} on hippocampal and hemispheric volumes was related to genotype; greater in APP/PS1, APP and PS1 than wild type mice. The CoQ_{10} appears to have a neuroprotective effect in aged mice. The protective effect was greater in mice which produce more amyloid plaques. The accumulation of β-amyloid aggregates in the brain plays a seminal role in the pathogenesis of AD [55]. While the neurotoxic mechanisms in the microenvironment of the plaque have not been fully elucidated, indirect evidence is consistent with a role for oxidative stress in the pathogenesis of MCI [56] and AD [22,34]. Previous studies have demonstrated a significant increase in oxidative stress in transgenic mice with β-amyloid pathology [1,3,14,21,37,39,45]. Additionally, increasing evidence suggests that oxidative stress/damage (amyloid beta peptide) lead to cell death through apoptosis or programmed cell death in AD [27,28,33]. Much neural loss will lead to brain atrophy. The microenvironment surrounding plaques is a local source of oxidative stress [4]. β-amyloid peptides can produce H_2O_2 [24] and provoke protein and lipid peroxidation [35]. It has a significant effect on neuronal DNA fragmentation, loss of neuritic networks, and cell viability [6,56]. Free radicals associated with plaques may mediate or contribute to plaque-induced toxicity [17,37,54].

To our best knowledge, this is the first time to demonstrate the neuroprotective effect of the diet supplement CoQ_{10} in brain volumes *in vivo*.

Our results showed an exogenous supplementation of the CoQ_{10}, a powerful antioxidant and free radical scavenger, delayed brain atrophy. The CoQ_{10} may act through its slowing oxidative stress-induced apoptosis and therefore delaying brain atrophy. Our present study indicates that a therapy with CoQ_{10} could be, in the future, a suitable strategy to delay MCI and AD progression.

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