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Original Research

Protective Effect of Edaravone Against $A\beta_{25-35}$-Induced Mitochondrial Oxidative Damage in SH-SY5Y Cells

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Abstract: Amyloid-β (Aβ)-induced oxidative stress plays an important role in the pathogenesis of Alzheimer's disease (AD). Recent studies show that Aβ accumulation may lead to mitochondrial oxidative damage. In the present study, we investigated the protective effect of edaravone on mitochondrial damage in SH-SY5Y cells treated with $A\beta_{25-35}$. SH-SY5Y cells were pre-treated with 20, 40 or 80 μM edaravone before treatment with 25 μM $A\beta_{25-35}$. After 24h cell culture, cellular apoptosis, intracellular reactive oxygen species (ROS), mitochondrial membrane potential (ΔΨm), ATP levels and mitochondrial morphology were evaluated. SH-SY5Y cells exposed to $A\beta_{25-35}$ had high levels of apoptosis and ROS; loss of ΔΨm, decreased ATP levels and presence of mitochondrial swelling. However, these effects were significantly inhibited by edaravone pre-treatment. These results indicate that edaravone prevents mitochondria oxidative damage caused by Aβ in SH-SY5Y cells, which suggests that it may have potential clinical application in AD therapy.

Key words: Edaravone; Alzheimer's disease; Amyloid-β peptide; Mitochondria damage.

Introduction

Alzheimer's disease (AD) is a complex and common nervous degenerative disease in the elderly. Patients with AD have brain dysfunction accompanied by behavioral and personality changes. AD is pathologically characterized by the presence of senile plaques formed by extracellular deposits of amyloid-β (Aβ) peptides and neurofibrillary tangles (NFTs) composed of intracellular aggregates of hyper-phosphorylated tau protein. Although the pathogenesis of AD remains unclear, a large number of studies have shown that genetic and epigenetic mechanisms are involved in its pathophysiology. In addition, Aβ-induced oxidative stress appears to play a key role in the pathogenesis and progression of this disease (1). Mitochondria are the major sources of intracellular ROS; mitochondrial dysfunction can increase ROS generation. Aβ has been reported to induce mitochondrial dysfunction and accelerate ROS production, thereby leading to cellular oxidative damage (2). In addition, studies have shown that while Aβ induces oxidative damage to mitochondria, the formation of Aβ is also enhanced by mitochondrial ROS, leading to a destructive self-perpetuating cycle (3, 4). Therefore, the disruption of this vicious circle and prevention of Aβ-induced oxidative damage to mitochondria could be a potential therapeutic approach to AD treatment.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent free radical scavenger which is popularly used as a neuroprotective agent in the treatment of acute ischemic stroke and acute hemorrhagic stroke (5). Previous studies have demonstrated that edaravone efficiently reduced mitochondrial damage in ischemia-reperfusion injury (6), acute renal injury (7), and retinal disease (8). However, not much is known about the possible protective effect of edaravone against Aβ-induced mitochondrial damage. Therefore, the present study was carried out to investigate if edaravone had a protective effect against mitochondria damage in AD, using a cell culture model of the disease established by treating SH-SY5Y cells with $A\beta_{25-35}$.

Materials and Methods

Cell culture and treatment

Human neuroblastoma SH-SY5Y cells from the cell bank of the Chinese Academy of Sciences were cultured in DMEM/F12 media (Gibco, USA) containing 10% FBS (HyClone, USA), 100 U/mL of penicillin and 100 μg/mL of streptomycin in an incubator at 37 °C in a medium containing 5% CO_2. In line with the protocol previously described by Wang *et al.* (9), $A\beta_{25-35}$ (Sigma, USA) was solubilized in distilled water at a concentration of 5 mM, incubated in a capped vial at 37 °C for 3 days to form aged $A\beta_{25-35}$ and stored frozen at −20 °C until use. Then SH-SY5Y cells were incubated with 25 μM $A\beta_{25-35}$ for 24 h to establish a cell culture model of AD. The experimental groups were (1) normal control group: SH-SY5Y cells were cultured for 27 h; (2) $A\beta_{25-35}$ group: after 3 h in control culture, SH-SY5Y cells were

treated with 25 μM $A\beta_{25-35}$ for 24 h; (3) edaravone + $A\beta_{25-35}$ group: SH-SY5Y cells were pre-treated with 20, 40 or 80 μM edaravone (Jida, China) for 3 h, and then $A\beta_{25-35}$ (at a final concentration of 25 μM) was added to the medium and incubated for additional 24 h.

Apoptosis assay

Cellular apoptosis was determined by flow cytometry. SH-SY5Y cells were cultured and treated as per the annexin V-FITC assay. This assay is based on the flow cytometry detection of the complex formed between green fluorescent probe FITC-labelled annexin V and phosphatidylserine residues on the surface of apoptotic cells. After treatment, the cells were washed twice in PBS, centrifuged at 2,000 rpm for 5 min, and then harvested. The cells were suspended in 500 μL of binding buffer and mixed with 5 μL of annexin V-FITC (KeyGen Biotech) and 5 μL of propidium iodide. The cells were then incubated at room temperature in the dark for 15 min. Flow cytometry was performed to detect apoptotic cells.

Measurement of intracellular ROS generation

Intracellular ROS were measured using an oxidation-sensitive fluorescent probe (DCFH-DA) (Beyotime Company, China). DCFH-DA itself is not fluorescent, but it can emit fluorescence when it is oxidized to form DCFH by ROS in cells. Thus, intracellular ROS can be detected indirectly by measurement of fluorescence intensity. After treatment, the cells were washed twice in phosphate-buffered saline (PBS), collected and adjusted to 1×10^7 cells/ml, and incubated with DCFH-DA at 37 °C for 20 min. The principle of this assay is that DCFH-DA is de-acetylated intracellularly by non-specific esterase, and is further oxidized by ROS to the fluorescent compound 2, 7-dichlorofluorescein (DCF). In this study, the fluorescence intensity of DCF was measured using a FACScan flow cytometer at an excitation wavelength (λ_{ex}) of 488 nm and an emission wavelength (λ_{em}) of 535 nm.

Measurement of mitochondrial membrane potential (ΔΨm)

The mitochondrial membrane potential (ΔΨm) of SH-SY5Y cells was measured using JC-1 according to the manufacturer's instructions (Beyotime Company, China). JC-1 is a fluorescent probe used to detect ΔΨm. It emits fluorescence of red and green when ΔΨm is increased and reduced, respectively. Therefore, the ratio of red to green fluorescence can be measured to reflect the level of ΔΨm. After treatment, cells were cultured in 6-well plates and incubated with JC-1 staining solution (5 μg/ml) for 20 min at 37 °C. The cells were then rinsed twice with JC-1 staining buffer, and the fluorescence intensity of mitochondrial JC-1 monomers (λ_{ex} 490 nm, λ_{em} 530 nm) and aggregates (λ_{ex} 525 nm, λ_{em} 590 nm) were detected using flow cytometry. The ΔΨm was calculated as the ratio of the intensity of the red fluorescence (i.e. aggregates) to that of the green fluorescence (i.e. monomers). Cells treated with 10 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP) were used as negative control. CCCP is a protonophore that can dissipate ΔΨm.

Measurement of cellular ATP levels

The levels of ATP in SH-SY5Y cells were determined using ATP Bioluminescence Assay Kit (Beyotime Company, China). Luciferin can be degraded by firefly luciferase to produce fluorescence, a process which is ATP-dependent. The amount of of fluorescence generated is proportional to the concentration of ATP in the presence of excess luciferase and luciferin. Thus the concentration of ATP can be estimated by measuring the fluorescence intensity. In this assay, the cells were collected and centrifuged at 12,000 × g for 5 min. The pellets were ground with 200 μL lysis buffer from the ATP detection kit. After centrifugation at 12,000 × g for 5 min at 4 °C, the supernatants were transferred to new tubes for testing. The level of ATP was determined by mixing 50 μl of the supernatant with 50 μl of luciferase reagent, which catalyzes light production from ATP and luciferin. The emitted light is linearly related to ATP concentration, and was measured using a microplate luminometer. ATP content was calculated based on a standard curve prepared using known concentrations of ATP (0.1–10 μM) diluted from the supplied standard solution.

Observation of mitochondrial morphology

Mitochondrial morphology in SH-SY5Y cells was observed by transmission electron microscopy (TEM). After appropriate treatments, the cells were harvested and washed twice with PBS. The cells were then pre-fixed with 2.5% glutaraldehyde, followed by immersion in 0.1 M phosphate buffer solution for 10 min. Thereafter, the cells were fixed in 1% osmium tetroxide at 4 °C for 2 h, and dehydrated with increasing concentrations of ethanol (50%, 60%, 70%, 80%, 90% and 100%), each for 15 min. They were subsequently stained overnight at room temperature with 2% uranyl acetate in 70% ethanol, and then embedded in Epon812. The embedded samples were sectioned into 60-nm-thick slices using a sliding ultramicrotome. The thin sections supported by copper grids were examined using a Hitachi TEM system operated at 100 kV.

Statistical analyses

All statistical analyses were performed using SPSS 11.0 software. All the data are expressed as mean ± SD. Significant differences were analyzed with Student's *t*-test and one-way analysis of variance (ANOVA), with the significance level set at $P < 0.05$.

Results

Cellular morphology changes under $A\beta_{25-35}$ treatment

SH-SY5Y cells were treated with 25 μM $A\beta_{25-35}$ and then cellular morphology was observed under the light microscope (×100). As shown in Plate 1A, cells in the normal control group showed clear cellular morphology, with a large number of axon terminals surrounding the body and no significant cell debris or aggregation. However, after exposure to $A\beta_{25-35}$ for 24 h, the cells assumed a wrinkled profile, and although the shortening of axons was reduced, there was a greater amount of cellular debris and aggregates (Plate 1B). It was exciting to

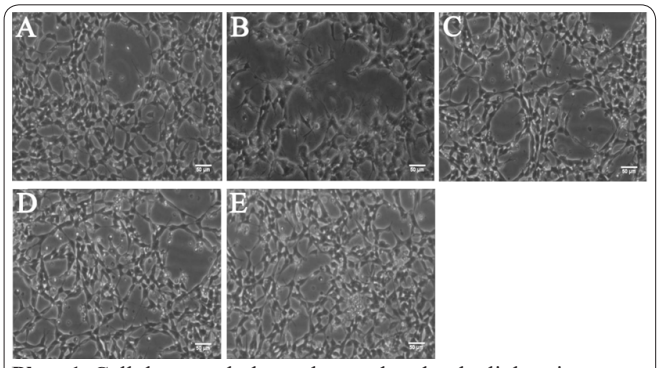

Plate 1. Cellular morphology observed under the light microscope (×100). A: image showing cells in the normal control group. B: Image of SH-SY5Y cells exposed to $A\beta_{25-35}$ for 24 h. C: Image showing morphology of cells pre-treated with 20 μM edaravone for 3 h and then exposed to $A\beta_{25-35}$ for 24 h. D: Image showing cells pre-treated with 40 μM edaravone for 3 h and then exposed to $A\beta_{25-35}$ for 24 h. E: Cells pre-treated with 80 μM edaravone for 3 h and then exposed to $A\beta_{25-35}$ for 24 h.

find that, compared to the $A\beta_{25-35}$-treated group, edaravone treatment protected the cells from $A\beta_{25-35}$-induced morphological changes. This indicated that edaravone might prevent cellular morphological alterations caused by $A\beta_{25-35}$ (Plates 1C, D, E).

Edaravone inhibited cellular apoptosis induced by $A\beta_{25-35}$

To investigate the reason for the increased cell debris caused by $A\beta_{25-35}$, cell apoptosis was measured in different treatment groups. As shown in Figure 1, the number of apoptotic cells was significantly increased in $A\beta_{25-35}$-treated group when compared to the control cells (64.6 vs. 6.3 %, p <0.05). However, the level of apoptosis in the three edaravone groups was significantly lower than that in the $A\beta_{25-35}$ group (47.3%, 25.3%, and 38.6%, compared to 64.6 %, p <0.05, Figure 1). Interestingly the middle dose (40 μM) of edaravone produced the highest protective effects against apoptosis induced by $A\beta_{25-35}$ (25.3% compared to 47.3% and 38.6 %, p <0.05, Figure 1). This indicates that although edaravone antagonizes cellular damage caused by $A\beta_{25-35}$, high concentration of edaravone alone may lead to cell death through a yet-to-be elucidated mechanism.

Edaravone reduced ROS accumulation induced by $A\beta_{25-35}$

In order to study the effects of edaravone on $A\beta_{25-35}$-induced generation of intracellular ROS, flow cytometry analysis was carried out using the ROS-sensitive fluorescence probe DCF. SH-SY5Y cells treated with 25 μM $A\beta_{25-35}$ for 24 h exhibited approximately 1.9-fold increase in fluorescence intensity relative to that of the normal control group. However, pre-treatment with edaravone for 3 h significantly decreased DCF fluorescence intensity when compared with the $A\beta_{25-35}$ group (p < 0.05). It is worthy of note that the ability of edaravone to suppress the intracellular ROS generation by $A\beta_{25-35}$ was maximum at a dose of 40 μM. Thus no protective effect was seen at the higher dose of 80 μM (Figure 2).

Edaravone antagonized $A\beta_{25-35}$-induced loss of mitochondrial membrane potential

Mitochondrial membrane potential (ΔΨm) is a key indicator of mitochondrial viability and function. ΔΨm in SH-SY5Y cells was measured by flow cytometry

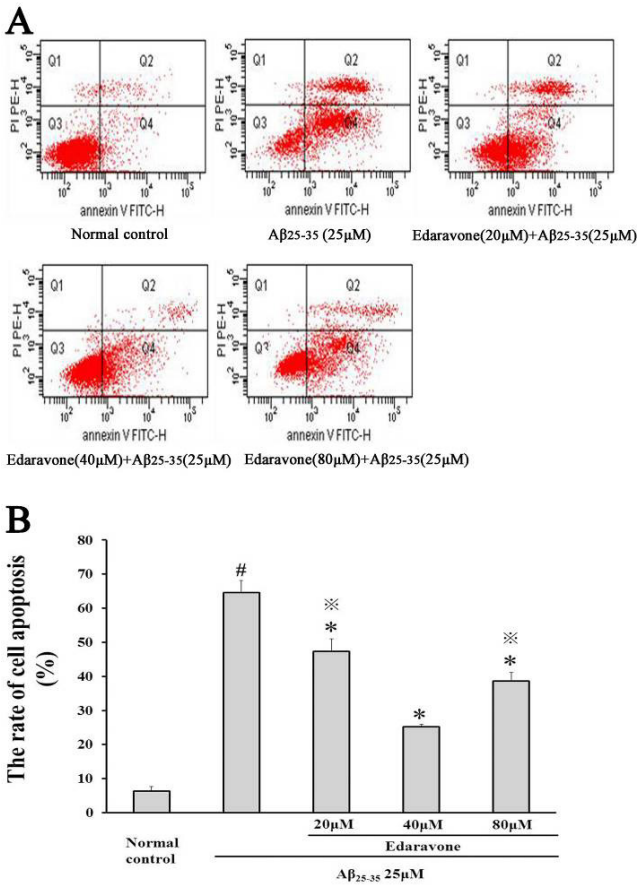

Figure 1. Effect of edaravone on apoptosis of $A\beta_{25-35}$-treated SH-SY5Y cells. SH-SY5Y cells, without or with edaravone pretreatment (20, 40, 80 μM) for 3 h, were exposed to $A\beta_{25-35}$ for 24 h, and then harvested and stained with annexin V-FITC and PI. They were then analyzed by flow cytometry (A). The level of cell apoptosis was calculated and analyzed in each group (B). All data are shown as mean ± SD of three independent experiments. $^{*}p$ < 0.05 relative to normal control group, $p^{\#}$ < 0.05 relative to $A\beta_{25-35}$ treatment alone, $^{※}p$ < 0.05 relative to 40 μM edaravone group.

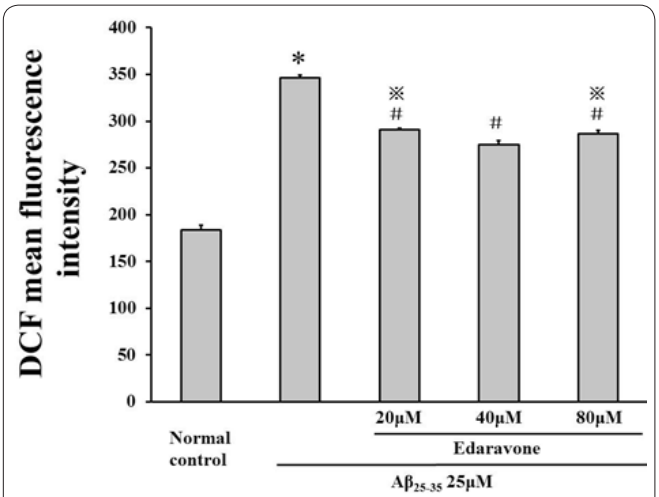

Figure 2. Protective effect of edaravone on $A\beta_{25-35}$-induced ROS generation in SH-SY5Y cells measured by flow cytometry. SH-SY5Y cells without or with edaravone pre-treatment (20, 40, 80 μM) for 3 h were exposed to $A\beta_{25-35}$ for 24 h, and the generation of intracellular ROS was measured with DCFH-DA. All data are shown as mean ± SD of three independent experiments. $^{*}p$ < 0.05 relative to normal control group, $^{\#}P$ < 0.05 versus $A\beta_{25-35}$ treatment alone, $^{※}p$ < 0.05 relative to 40 μM edaravone group.

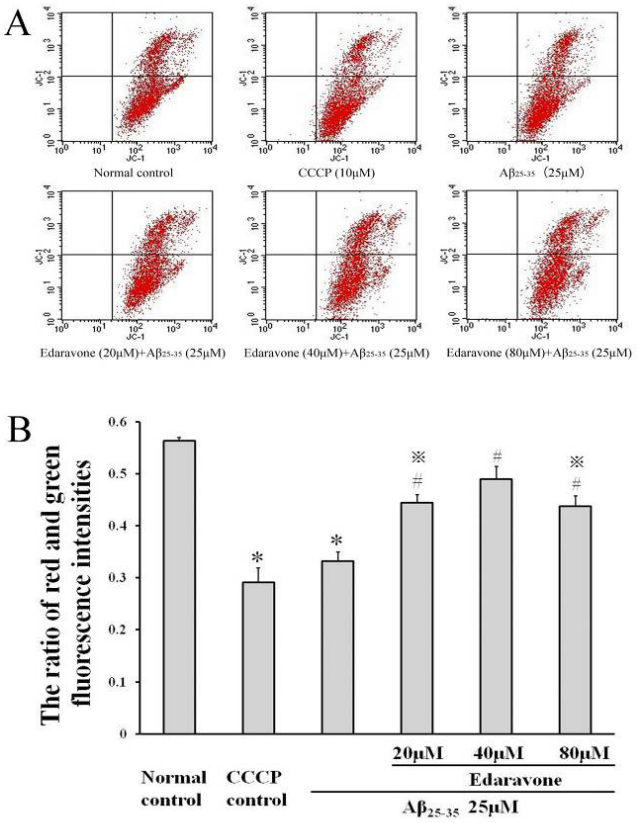

Figure 3. Edaravone prevented $A\beta_{25\text{-}35}$-induced loss of mitochondrial membrane potential ($\Delta\Psi m$). SH-SY5Y cells, without or with edaravone pre-treatment (20, 40, 80 μM) for 3 h, were exposed to $A\beta_{25\text{-}35}$ for 24 h, and then membrane potential was evaluated by flow cytometry using JC-1 (A). The ratio of red to green fluorescence intensity was calculated and analyzed in each group (B). All data are shown as mean ± SD of three independent experiments. $^{*}p < 0.05$ relative to normal control group, $^{\#}p < 0.05$ relative to $A\beta_{25\text{-}35}$ treatment alone, $p < 0.05$ relative to 40 μM edaravone group.

with the fluorescent probe JC-1. Compared to the normal control group, the ratio of red to green fluorescence intensity was markedly reduced ($p < 0.05$) in SH-SY5Y cells treated with 25 μM $A\beta_{25\text{-}35}$ for 24 h, indicating a drop in $\Delta\Psi m$ and mitochondrial dysfunction. Edaravone pre-treatment (20, 40, 80 μM) significantly inhibited the $A\beta_{25\text{-}35}$-induced loss of $\Delta\Psi m$ in SH-SY5Y cells ($p < 0.05$). However, $\Delta\Psi m$ in SH-SY5Y cells pre-treated with 40 μM edaravone was significantly higher than that in cells treated with 20 or 80 μM edaravone ($p < 0.05$), indicating that among the doses tested, 40 μM had an optimal protective effect against $A\beta_{25\text{-}35}$-induced loss of $\Delta\Psi m$ (Figure 3).

Edaravone suppressed $A\beta_{25\text{-}35}$-induced reduction in ATP levels

Cellular ATP level is also a sensitive index of mitochondrial function. Therefore, to further clarify the effects of edaravone on mitochondrial dysfunction induced by $A\beta_{25\text{-}35}$ in SH-SY5Y cells, cellular ATP levels were measured. ATP levels were significantly reduced in SH-SY5Y cells exposed to 25 μM $A\beta_{25\text{-}35}$ for 24 h when compared with the normal control group ($p < 0.05$). Pre-treatment with edaravone (20, 40, 80 μM) significantly improved cellular ATP levels when compared with cells treated with $A\beta_{25\text{-}35}$ alone ($p < 0.05$). Moreover, ATP levels in the 40 μM edaravone group were significantly higher than ATP levels in the 20 and 80 μM edaravone groups ($p < 0.05$) (Figure 4).

Edaravone protected against $A\beta_{25\text{-}35}$-induced changes in mitochondrial morphology

The preceding results indicate that edaravone protected against mitochondrial dysfunction in SH-SY5Y cells exposed to $A\beta_{25\text{-}35}$, and that 40 μM of the drug had an optimal protective effect. To assess whether this protective effect was associated with the maintenance of mitochondrial morphological integrity, SH-SY5Y cells, with or without pre-treatment with 40 μM edaravone for 3 h, were exposed to $A\beta_{25\text{-}35}$ for 24 h and then examined by TEM. In SH-SY5Y cells treated with $A\beta_{25\text{-}35}$, mitochondria were significantly reduced in number, and there was obvious swelling of the remaining mitochondria, as well as vacuolation and reduction or disappearance of mitochondrial cristae (Plates 2C and 2D). These deleterious changes were absent in the normal control group (Plates 2A and 2B). However, pre-treatment with 40 μM edaravone produced a significant reduction in mitochondrial swelling, inhibited the disappearance of mitochondrial cristae, and reduced vacuolation in SH-SY5Y cells exposed to $A\beta_{25\text{-}35}$ (Plates 2E and 2F).

Discussion

Numerous studies have demonstrated that Aβ produces mitochondrial dysfunction and increases ROS levels, and that mitochondrial ROS enhance the amyloidogenic processing of amyloid precursor protein into Aβ, thereby generating a self-perpetuating cycle that further impairs mitochondrial function (2,10). Therefore, mitochondria-targeting antioxidants that prevent Aβ-induced oxidative damage may have substantial efficacy in AD treatment. In addition to its application in the treatment of cerebral infarction, edaravone has been

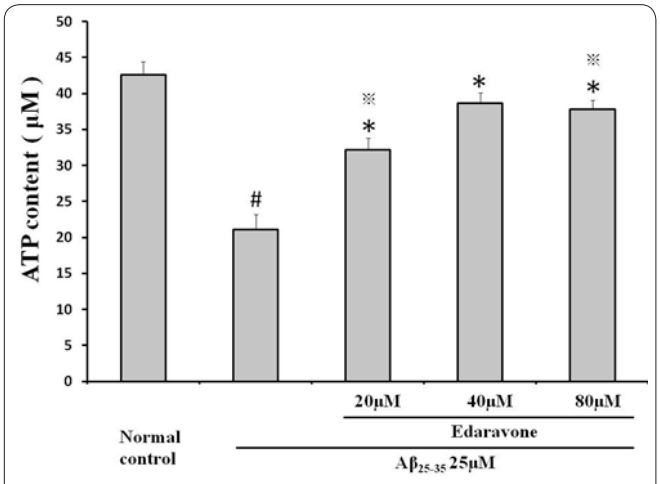

Figure 4. Effect of edaravone on cellular ATP content in SH-SY5Ycells exposed to $A\beta_{25\text{-}35}$. SH-SY5Y cells, without or with edaravone pre-treatment (20, 40, 80 μM) for 3 h, were exposed to $A\beta_{25\text{-}35}$ for 24 h, and then cellular ATP content was measured using a luminometer. The results are expressed as mean ± SD of three independent experiments ($^{\#}p < 0.05$ relative to normal control group, $^{*}p < 0.05$ relative to $A\beta_{25\text{-}35}$ treatment alone, $^{※}p < 0.05$ relative to 40 μM edaravone group).

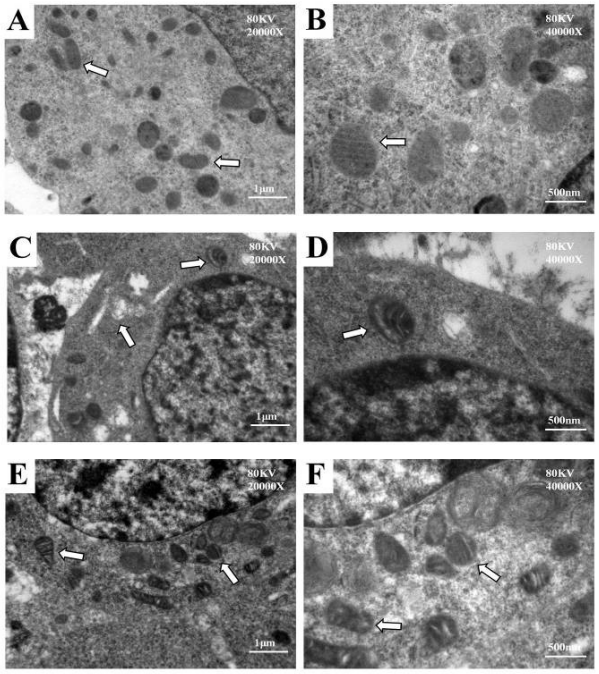

Plate 2. Effect of edaravone on morphological changes in mitochondria in SH-SY5Y cells exposed to $A\beta_{25-35}$. SH-SY5Y cells, without or with pre-treatment with 40 μM edaravone for 3 h, were exposed to $A\beta_{25-35}$ for 24 h and then observed using TEM. A and B are TEM images showing normal appearance of mitochondria (indicated by arrows) in the normal control group. C and D depict mitochondrial damage characterized by mitochondrial swelling, disappearance of mitochondrial cristae and vacuolation (indicated by arrows) in cells treated with $A\beta_{25-35}$ (25 μM) alone. E and F show nearly normal appearance of mitochondria (indicated by arrows) in cells pre-treated with 40 μM edaravone (x20,000 and x40,000).

found to protect neurons in a number of other neurological diseases (8,11). Results from our previous investigations, and from studies elsewhere suggest that edaravone alleviates oxidative damage in AD by scavenging free radicals (12,13). However, the protective effect of edaravone against Aβ-induced oxidative damage to mitochondria had not been investigated.

Since mitochondria are vulnerable to oxidative stress, and since it is known that Aβ can induce oxidative stress by increasing the production of ROS, the present study examined the effects of edaravone on the generation of intracellular ROS. It was found that ROS levels were increased approximately 1.9-fold in SH-SY5Y cells exposed to $A\beta_{25-35}$ when compared with control cells. Interestingly, edaravone pre-treatment significantly reduced the generation of intracellular ROS in SH-SY5Y cells exposed to $A\beta_{25-35}$. Mitochondria are the major producers of ROS, and at the same time, major targets of ROS toxicity. These findings and those of Yan and colleagues (4, 14) indicate that edaravone has the ability to inhibit the generation of ROS induced by Aβ, thereby attenuating oxidative damage to mitochondria, as well as reducing mitochondrial ROS generation these. These protective effects of edaravone contribute significantly to the maintenance of mitochondrial function and cell viability.

Several studies have shown that Aβ not only increases ROS production, but also induces mitochondrial dysfunction, including loss of mitochondrial membrane potential and a reduction in ATP levels (15,16). Aβ may inhibit ATP generation by interacting with the alpha subunit of ATP synthase or by decreasing cytochrome c oxidase activity (15,17,18). This triggers progressive loss of mitochondrial membrane potential through the opening of mitochondrial permeability transition pores or by activation of poly-ADP-ribose polymerase (19). In agreement with these studies showing that Aβ induces mitochondrial dysfunction, the present study demonstrated that ATP levels and mitochondrial membrane potential were reduced in SH-SY5Y cells exposed to 25 μM $A\beta_{25-35}$ for 24 h, and that these changes were significantly inhibited by edaravone pre-treatment, with 40 μM edaravone having an optimal effect. Previous *in vitro* and *in vivo* experiments have shown that edaravone markedly attenuated the loss of mitochondrial membrane potential and the reduction in ATP levels in a variety of disease models, including ischemia-reperfusion injury and acute renal damage (6, 20 - 22). Results from the present study show clearly that edaravone pre-treatment also protected against loss of mitochondrial membrane potential and the reduction in ATP levels in a cell culture model of AD established by exposing SH-SY5Y cells to $A\beta_{25-35}$. Since the ATP generated by mitochondria participates in many cellular functions, such as intracellular calcium regulation, neurotransmitter production and synaptic plasticity, the loss of mitochondrial membrane potential is considered an early indicator of apoptosis. Therefore, it is reasonable to suggest that edaravone may not only prevent mitochondrial damage, but also protect against apoptosis induced by $A\beta_{25-35}$. In normal conditions, intracellular ROS is an key molecule for cell proliferation and differentiation (23, 24). In this study, it was found that while 80μM edaravone could scavenge intracellular ROS effectively, it led to inbalance between oxidants and antioxidants and disturbance of mitochondrial function. Thus, to some extent, 80μM edaravone might have some cytotoxic effects.

In addition, we found that mitochondrial swelling and vacuolation were significantly ameliorated in SH-SY5Y cells pre-treated with edaravone when compared with SH-SY5Y cells treated with $A\beta_{25-35}$ alone. In an ischemia-reperfusion model, Yamamura *et al.* found that pre-treatment with edaravone alleviated the ischemia reperfusion-induced disruption of mitochondrial structure (6, 25). In the present study, results show that edaravone attenuated mitochondrial swelling induced by $A\beta_{25-35}$, thereby providing further support for a protective role of the drug in this cell culture model of AD.

Previous studies have shown that excessive ROS can increase intracellular calcium influx mediated by voltage-gated calcium channels (VGCC) and incresed Ca^{2+} influx, thereby causing calcium overload. Edaravone can remove excess ROS and help to reduce intracellular calcium. Moreover, He *et al.* have found that edaravone blocked Aβ-induced opening of PC12 cells VGCC and inhibited calcium overload (26). Thus further investigations are necessary to find out whether the protective effect of edaravone on mitochondria is related to antagonism of calcium channels.

Similar to *Ginkgo biloba* extract EGb761, the antioxidant effect of edaravone has been explored in the treatment of acute stroke. Some researchers believe that EGb761 may be effective in the treatment and prevention of AD. However, some studies reported that high

doses of EGb761 exacerbated cellular oxidative damage and apoptosis. Thus the clinical efficacy of EGb761 in the management of AD remains elusive. Multiple factors such as population sensitivity, severity of impairment and dose interfere with the clinical efficacy of EGb761. In addition, EGb761 has multiple components (24% flavonoid glycosides, 6% terpenoids and organic acids) and it is involved in many pathological processes such as oxidative stress, inflammation, apoptosis and calcium overload (27, 28). Thus EGb761 does not exert a specific unidirectional action (activation or inhibition) in various domains in AD physiology and pathology; rather its role is regulatory (29). The involvement of EGb761 in multiple processes, and its multi-component nature may make its effect in AD treatment less obvious. In contrast, edaravone is a monomeric drug, and so its pharmacological effects are much clearer. It is used for treating different diseases of diverse aetiologies, based on its free radical scavenging properties. These differences between EGb761 and edavarone may account for differences in their different treatment effects.

AD pathogenesis involves multiple processes which are not yet fully elucidated. The present study was conducted *in vitro* using SH-SY5Y cells in order to directly observe the protective effect of edaravone on Aβ-induced mitochondrial damage in the AD cell model. Recently, Yang *et al.* used the rat model to validate the effects of edaravone on reduction of cognitive dysfunction in AD (30). This provides an experimental basis for further clarification of the mechanism of action of edaravone against AD.

However AD models have certain limitations. For instance, the models cannot completely simulate the complex pathology in AD patients. This is considered a deficiency in this study.

The results obtained in this study demonstrate that edaravone protects against $A\beta_{25-35}$-induced oxidative damage to mitochondria in SH-SY5Y cells by attenuating ROS production, mitochondrial swelling, loss of mitochondrial membrane potential and reduction in ATP levels. These findings suggest that the antioxidant edaravone may have promising therapeutic efficacy in AD through a mechanism involving blockage of mitochondrial dysfunction.

Acknowledgments

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