Neurochem Res
DOI 10.1007/s11064-015-1623-0

ORIGINAL PAPER

The Neuroprotective Effects of Decursin Isolated from *Angelica gigas* Nakai Against Amyloid β-Protein-Induced Apoptosis in PC 12 Cells *via* a Mitochondria-Related Caspase Pathway

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Received: 6 March 2015 / Revised: 19 May 2015 / Accepted: 20 May 2015

Abstract Decursin, purified from *Angelica gigas* Nakai, has been proven to exert neuroprotective property. Previous study revealed decursin protected the PC12 cells from $A\beta_{25-35}$-induced oxidative cytotoxicity. The present study aimed to investigate whether decursin could protect PC12 cells from apoptosis caused by Aβ. Our results indicated that pretreatment of PC12 cells with decursin significantly inhibited $A\beta_{25-35}$-induced cytotoxicity and apoptosis. The mechanism of action is likely to reverse $A\beta_{25-35}$-induced mitochondrial dysfunction, including the reduction of mitochondrial membrane potential, the inhibition of reactive oxygen species production, and the decrease of mitochondrial release of cytochrome *c* in PC12 cells. In addition, decursin significantly suppressed the activity of caspase-3 and moderated the ratio of Bcl-2/Bax induced by $A\beta_{25-35}$. These findings indicate that decursin exerts a neuroprotective effect against $A\beta_{25-35}$-induced neurotoxicity in PC12 cells, at least in part, via suppressing the mitochondrial pathway of cellular apoptosis.

Keywords Decursin · Alzheimer's disease · Amyloid β · Apoptosis

Introduction

Alzheimer's disease (AD) is the most common form of senile dementia, affecting millions of people worldwide [11]. The deposition of β-amyloid (Aβ) within the senile plaques which is a hallmark of AD has been considered to play an important role in the development and progression of AD [12, 33]. Elevation of oxidative stress and activation of the apoptotic pathway play key roles in mediating Aβ induced toxicity and neural death [2, 9, 27, 32]. Thus, an approach which simultaneously intervene the neuronal apoptosis may be effective in combating neurodegeneration.

Angelica gigas Nakai (Umbelliferae) is used in traditional oriental herbal medicine for the treatment of gynecological diseases such as menoxenia. Various pharmacological properties, including anti-cancer [35], anti-bacterial [23], anti-platelet aggregation [24], and anti-nematodal [34] activities, anti-allergic effect [13], anti-amnestic activity [17] are generally attributed to the major active ingredient decursin [1, 6]. The majority of clinical studies on the use of antioxidants as neuroprotective agents had very limited success, primarily due to the impermeability of the blood–brain-barrier (BBB) to most of the compounds investigated. However, the pioneering work of Madgula et al. identified that decursin has the potential to cross the BBB and penetrate the central nervous system (CNS) [28]. Decursin exhibits potent neuroprotective

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Published online: 16 June 2015

activity in vitro [16] and in vivo [15]. Previous study also found that decursin has anti-amnesic effects against Aβ [14]. These observations support the use of decursin in the treatment of CNS disorders including AD. Our previously study indicated that decursin augments cellular antioxidant defense capacity through both intrinsic free radical scavenging activity and activation of MAPK signal pathways that leads to Nrf2 activation, and subsequently HO-1 induction, thereby protecting the PC12 cells from $A\beta_{25-35}$-induced oxidative cytotoxicity [26]. However, whether decursin has protective effect on Aβ-induced apoptosis has not yet been reported. As a part of our program to evaluate the neuroprotective activity of decursin, we sought to determine whether decursin could protect PC12 from apoptosis caused by Aβ.

Materials and Methods

Materials

Amyloid beta-protein (25–35) trifluoroacetate salt ($A\beta_{25-35}$) was provided by Bachem (Torrence, CA). RPMI + GlutaMAX^{TM}-l, penicillin-streptomycin, fetal bovine serum (FBS) and horse serum (HS) were purchased from Invitrogen (Grand Island, NY). BCA^{TM} protein assay kit was purchased from ThermoFisher Scientific (Barrington, IL). The assay kit for cytotoxicity (WST-8) was supplied by Cayman Chemical Company (Ann Arbor, MI). Antibodies to cytochrome *c* rabbit mAb, Bcl-2 rabbit mAb, Bax rabbit mAb, glyceraldehydes-3-phosphate dehydrogenase (GAPDH) rabbit mAb, and anti-rabbit IgG alkaline phosphatase (AP)-linked antibodies were obtained from Cell Signaling Technology (Danvers, MA). All the other reagents were of the highest grade and were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise indicated.

Preparation of Decursin

Decursin was prepared by Dr. M.-J. Kim's lab in the Department of Smart Foods and Drugs, Inje University, as described previously [18, 19]. Briefly, dried and powdered root of *A. gigas* Nakai (1 kg) was extracted with 5 L of 95 % ethanol for 24 h at room temperature. Extracts were filtered through Whatman No. 1 filter paper, and were concentrated using a rotary evaporator (R-200, Büchi Labortechnik AG, Flawil, Switzerland) under reduced pressure, and 50 g *A. gigas* Nakai ethanol extract (AGNEX) was obtained. Decursin was purified from AGNEX using recycling preparative HPLC (LC-9104, JAI, Tokyo, Japan). The AGNEX (20 g) was dissolved in 30 mL of 70 % acetonitrile/water and filtered with a 0.45 μm membrane filter. 3 mL of sample was injected to the JAIGEL ODS-AP column (20 × 500 mm, JAI) at a flow rate of 4 mL/min. Isocratic elution was applied using 70 % acetonitrile/water as the mobile phase, and the peaks were detected using a RI and UV/Vis detector at 328 nm. Finally, 5.3 g of decursin was obtained.

Preparation of $A\beta_{25-35}$ Stock Solution

$A\beta_{25-35}$, the most toxic peptide fragment derived from the amyloid precursor protein, was dissolved in deionized distilled water at a concentration of 1 mM, and was incubated in a 37 °C for 72 h to induce maximal aggregation according to the previous report [39]. The solution was stored at −80 °C to create stable conditions for the aged stock solution, and diluted in serum-free medium to desired concentrations immediately before use.

Cell Culture

The rat pheochromocytoma (PC12) cells were maintained in RPMI + GlutaMAX^{TM}-l supplemented with 5 % FBS, 10 % HS, and 1 % penicillin-streptomycin and cultured at 37 °C in a humidified atmosphere of 5 % CO_2. All cells were plated in poly-*L*-lysine coated culture dishes. The medium was changed every other day, and the cells were plated at an appropriate density according to the scale of each experiment. After the cells were attached, they were switched to serum-free medium for treatment. The cells were incubated with and without $A\beta_{25-35}$ for 24 h, followed by exposure to various concentrations of decursin which were diluted in serum-free medium for 3 h. Data were from three experiments with triplicates in all cases.

Cell Viability Assay

The cell viability was assessed by the WST-8 cell proliferation assay kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI). Briefly, PC12 cells were seeded in 96-well culture plates. After incubation, the media was supplemented with 10 μL/well WST for 2 h prior to spectrophotometric evaluation. Conversion of WST to formazan was measured at 450 nm by fluorescence multi-detection reader (Synergy HT, Biotek, Highland Park, IL). This reaction reflects the reductive capacity of the cells, representing the viability of the cells, and the results were expressed as the percentage of control (untreated) cells. Decreased WST reduction was taken as an indication of neuronal cell injury.

Apoptotic Rates Assessment

The apoptosis rates were measured by propidium iodide (PI). Following treatment, PC12 cells were collected and fixed with 70 % ice-cold ethanol for 1 h. Then all the

samples were dyed with PI. The apoptotic rates were measured with a FACSCalibur flow cytometer (BD Biosciences).

Intracellular Reactive Oxygen Species (ROS) Measurement

ROS production in PC12 cells was measured using the redox-sensitive fluorescent dye H_2DCF-DA. Briefly, PC12 cells were seeded in 96-well plates, following treatment, the cells were loaded with 10 μM H_2DCF-DA at 37 °C for 30 min in the dark, and then washed twice with PBS. Finally, the fluorescence intensity was measured at the excitation wavelength of 485 nm and the emission wavelength of 530 nm using a fluorescence microplatereader (Synergy HT, Biotek, Highland Park, IL). Data were analyzed and expressed as a percentage of the control.

Mitochondrial Membrane Potential (MPP) Analysis

To measure MPP, the fluorescent dye rhodamine 123 (Rh123), a cell-perme-able cationic dye that preferentially enters into mitochondria based on the highly negative MPP, was used. Depolarization of MPP results in the loss of Rh123 from the mitochondria and a decrease in intracellular fluorescence. Briefly, PC12 cells were seeded in 96-well plates. Following treatment, the cells were added 10 mM Rh123 for 30 min at 37 °C, and then washed twice with PBS. The fluorescence intensity was evaluated at the excitation wavelength of 488 nm and the emission wavelength of 510 nm with a fluorescence microplate reader (Synergy HT, Biotek, Highland Park, IL). Data were analyzed and expressed as a percentage of the control.

Determination of Caspase-3 Activity

The caspase-3 activity was measured using a colorimetric assay kit according to the manufacturer's protocol (Sigma-Aldrich, St Louis, MO, USA). Briefly, after treatment, the PC12 cells were washed with D-Hanks solution. Then, the cells were incubated with 500 μL of lysis buffer on ice for 30 min. The cells were harvested by centrifugation at 16,000×g for 10 min at 4 °C. The supernatant was incubated with the substrate (acetyl-Asp-Glu-Val-Asp-p-nitroanilide) at 37 °C for 90 min. The activity of caspase-3 was monitored spectrophotometrically at 405 nm. Data were analyzed and expressed as a percentage of the control.

Cytosolic Lysate Preparation and Western Blot Analysis

Cells were treated with various reagents as detailed in the figure legends. All steps were carried out on ice or at 4 °C unless stated otherwise. Protease inhibitors (10 μg/mL aprotonin, 10 μg/mL leupeptin) and a reducing agent (1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) were added to lysis buffer just prior to use. Briefly, after treatment the cells were washed and collected with PBS. Total proteins from different groups were extracted by using cell lysis buffer. Lysates were centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was kept as the cytoplasmic extract and stored at −70 °C until use. The protein concentrations were measured with a BCA™ protein assay kit (ThermoFisher Scientific, Barrington, IL).

Western blot was performed by the standard method. Equal amounts of proteins were fractionated by 10 % SDS-polyacrylamide gel electrophoresis and electro-transferred to an Immun-Blot™ PVDF membrane (0.2 μM pore size, Bio-Rad). Membranes were blocked overnight at 4 °C in Tris-buffered saline (TBS), 0.05 % (v/v) Tween-20, 150 mM NaCl, and 5 % (w/v) Bovine Serum Albumin (BSA, Santa Cruz Biotechnology, Santa Cruz, CA), followed by 2 h incubation with primary antibody diluted in the same buffer (cytochrome c 1:1000, Bcl-2 1:1000, Bax 1:1000, and GAPDH 1:1000). After washing with 0.1 % (v/v) Tween-20 in TBS, the membrane was incubated with anti-rabbit IgG AP-linked secondary antibody for 1 h at room temperature and then washed with the same buffer. The immune-blotted membrane was developed with 5-bromo-4-chloro-3-indoyl phosphate (BCIP)/nitrobluetetrazolium (NBT) color-developing solution. The blots in the samples were quantified by densitometry analysis using PDQuest software (version 7.0, Bio-Rad, Hercules, CA). All data from three independent experiments were expressed as the relative intensity compared to the control group for the statistical analyses.

Statistical Analysis

Data were represented as the mean ± SD (standard deviations) from three experiments with triplicates in all cases. Statistical analyses were performed using one-way analysis variance (ANOVA) followed by Dunnet's post hoc test to express the difference among the groups. Data considered statistically significant at $p < 0.05$.

Results

Effect of Decursin on Cell Viability in PC12 Cells

Initially, the cytotoxic potential of decursin on PC12 cells was measured. As shown in Fig. 1a, no cytotoxic effects of decursin were observed up to a concentration of 10 μM. However, higher amount decursin reduced the viability of the PC12 cells. Furthermore, 10 μM decursin treatment for

24, 48 and 72 h did not show any toxic effect on PC12 cells (Fig. 1b). Thus, for further experiments, the cells were treated with decursin in the concentration range of 0.01–10 μM.

Decursin Attenuated Aβ$_{25–35}$-Induced Cytotoxicity in PC12 Cells

To test the neuroprotective effect of decursin, we tested its protective effect on Aβ$_{25–35}$-induced cytotoxicity in PC12 cells. PC12 cells were treated with various sub-lethal concentrations of decursin for 3 h, followed by further incubation in the presence or the absence of Aβ$_{25–35}$ for 24 h. Treatment with Aβ$_{25–35}$ (25 μM) for 24 h induced approximately 40 % cell death, whereas pretreatment with decursin (0.01–10 μM) diminished the cell death in response to Aβ$_{25–35}$ (Fig. 2).

Decursin Prevented Aβ$_{25–35}$-Induced Apoptosis in PC12 Cells

The apoptosis of PC12 cells induced by Aβ$_{25–35}$ was investigated with flow cytometry. As shown in Fig. 3, pretreatment with decursin (0.01–10 μM) could effectively prevent the increase of cell apoptosis induced by Aβ$_{25–35}$.

Decursin Inhibited Intracellular ROS Generation in Aβ$_{25–35}$-induced PC12 Cells

ROS production can be used as a marker of mitochondrial dysfunction. To investigate whether decursin could prevent Aβ$_{25–35}$-induced ROS generation in PC12 cells, we measured the intracellular ROS production by using the fluorescence assay with H_2DCF-DA probe. As shown in Fig. 4, exposure of the cells to 25 μM Aβ$_{25–35}$ for 24 h significantly increased the intracellular ROS level. Pretreatment with decursin (0.01–10 μM) markedly reduced the ROS generation in PC12 cells.

Effect of Decursin on the Expression of Proapoptotic Proteins in Aβ$_{25–35}$-Induced PC12 Cells

To investigate the possible antiapoptotic effect of decursin on Aβ$_{25–35}$-induced cell death, the protein levels of Bax and Bcl-2 were determined by western blot analysis. As illustrated in Fig. 5, Bax protein levels were increased and Bcl-2 protein levels were decreased in Aβ$_{25–35}$ treated PC12 cells. Pretreatment with decursin blocked the upregulation of Bax and downregulation of Bcl-2 by Aβ$_{25–35}$ treatment. The data indicated that decursin pretreatment partially inhibited the reduction of the Bcl-2/Bax ratio observed after Aβ$_{25–35}$ treatment. These results further demonstrate that decursin has protective effects against Aβ$_{25–35}$-induced apoptosis.

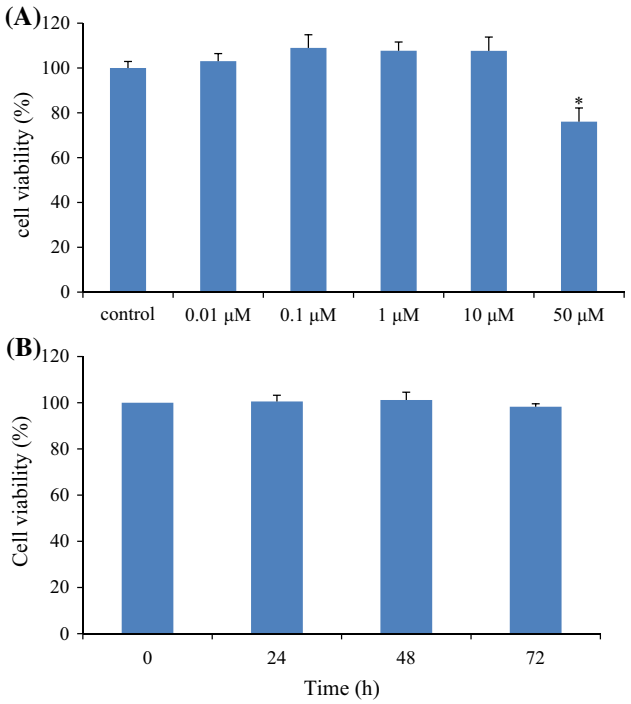

Fig. 1 Effect of decursin on cell viability of PC12 cells. **a** PC12 cells were incubated with various concentrations of decursin (0.01–50 μM) for 24 h. **b** PC12 cells were incubated with 10 μM decursin for 24, 48 and 72 h. Cell viability was estimated by the WST-8 assay. Data are expressed as percent of values in untreated control cultures. *$p < 0.05$ compared with control

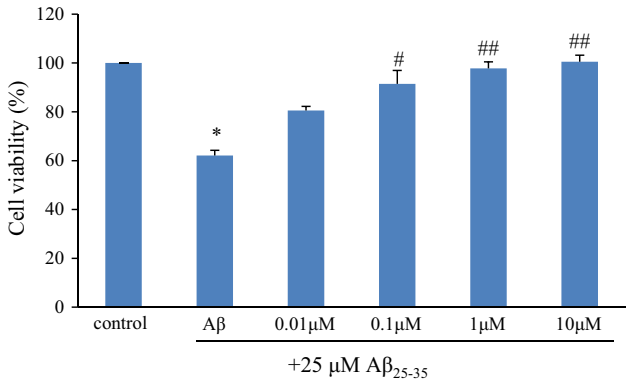

Fig. 2 Effect of decursin on Aβ$_{25–35}$-induced neurotoxicity in PC12 cells. PC12 cells were pretreated with various concentrations of decursin for 3 h and then incubated with and without 25 μM Aβ$_{25–35}$ for 24 h. Cell viability was estimated by the WST-8 assay. Data are expressed as percent of values in untreated control cultures. *$p < 0.05$ compared with control. $^{\#}p < 0.05$ compared with the group treated by Aβ$_{25–35}$ alone. $^{\#\#}p < 0.01$ compared with the group treated by Aβ$_{25–35}$ alone

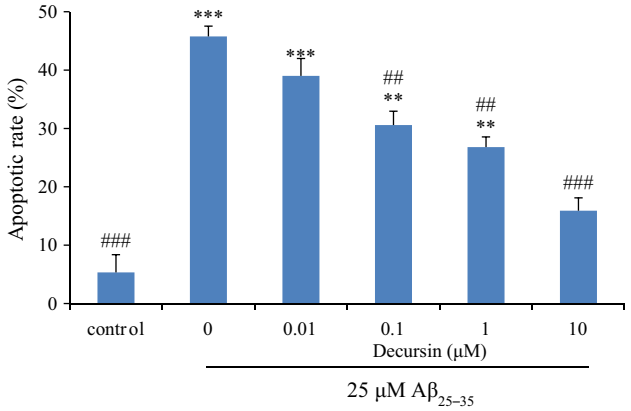

Fig. 3 Effect of decursin on $A\beta_{25–35}$-induced neurotoxicity in PC12 cells. PC12 cells were pretreated with various concentrations of decursin for 3 h and then incubated with and without 25 μM $A\beta_{25–35}$ for 24 h. Apoptotic rate was estimated by PI. $^{**}p < 0.01$ compared with control. $^{***}p < 0.001$ compared with control. $^{\#\#}p < 0.01$ compared with the group treated by $A\beta_{25–35}$ alone. $^{\#\#\#}p < 0.01$ compared with the group treated by $A\beta_{25–35}$ alone

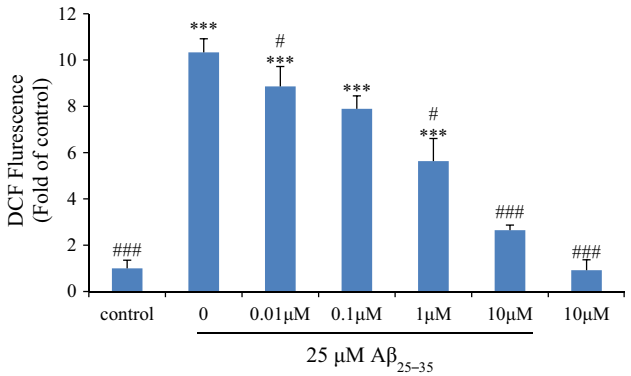

Fig. 4 Effect of decursin on intracellular ROS generation in $A\beta_{25–35}$-induced PC12 cells. PC12 cells were pretreated with various concentrations of decursin for 3 h and then incubated with and without 25 μM $A\beta_{25–35}$ for 24 h. After that the cells were washed with PBS and then treated with 10 μM H_2DCF-DA for 30 min. Intracellular ROS production was measured at the excitation wavelength of 485 nm and the emission wavelength of 530 nm using the fluorescence microplate reader. $^{***}p < 0.001$ compared with control. $^{\#}p < 0.05$ compared with the group treated by $A\beta_{25–35}$ alone. $^{\#\#\#}p < 0.001$ compared with the group treated by $A\beta_{25–35}$ alone

Decursin Reversed the MMP in $A\beta_{25–35}$-Induced PC12 Cells

The effect of decursin on MMP in $A\beta_{25–35}$-treated PC12 cells is shown in Fig. 6. Treatment of PC12 cells with 25 μM $A\beta_{25–35}$ for 24 h caused a significant dissipation of MMP. Pretreatment with various concentrations of decursin to $A\beta_{25}$_35-treated PC12 cells significantly improved $A\beta_{25–35}$-induced impairments in MMP. These results

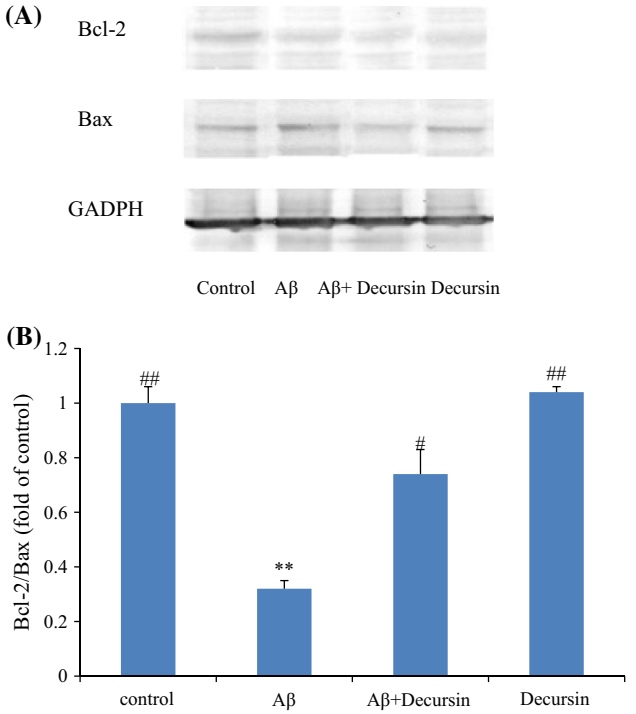

Fig. 5 Effect of decursin on the expression of proapoptotic proteins in $A\beta_{25–35}$-induced PC12 cells. PC12 cells were pretreated with 10 μM decursin for 3 h and then incubated with and without 25 μM $A\beta_{25–35}$ for 24 h, after that the Bcl-2 and Bax protein levels were determined by western blot analyses. Data are expressed as fold of control. $^{**}p < 0.01$ compared with control. $^{\#}p < 0.05$ compared with the group treated by $A\beta_{25–35}$ alone. $^{\#\#}p < 0.01$ compared with the group treated by $A\beta_{25–35}$ alone

suggested that decursin attenuates $A\beta_{25–35}$-induced mitochondrial dysfunction.

Decursin Attenuated the Release of Cytochrome *c* in $A\beta_{25–35}$-Induced PC12 Cells

As shown in Fig. 7, $A\beta_{25–35}$ caused a significant enhancement of the cytochrome *c* release to the cytosol after 24 h treatment. Pretreatment of PC12 cells by decursin significantly attenuated the $A\beta_{25–35}$-induced release of cytochrome *c* into the cytosol.

Decursin Depressed Caspase-3 Activity in $A\beta_{25–35}$-Induced PC12 Cells

Caspase-3 is the final executor of apoptosis. Results (Fig. 8) showed that caspase-3 activities were increased by application of $A\beta_{25–35}$ for 24 h. Pretreatment with decursin significantly inhibited caspase-3 activities in a concentration-dependent manner. These results indicate that decursin may inhibit $A\beta_{25–35}$-induced apoptosis in PC 12 cells via de-activation of the caspase apoptotic pathway.

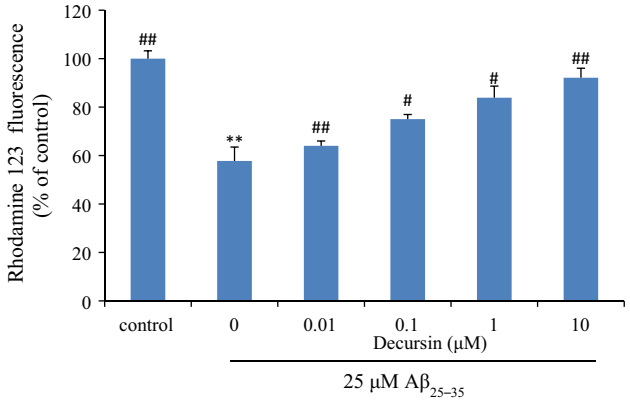

Fig. 6 Effect of decursin on the mitochondrial membrane potential (MMP) in $A\beta_{25–35}$-induced PC12 cells. PC12 cells were pretreated with various concentrations of decursin for 3 h and then incubated with and without 25 μM $A\beta_{25–35}$ for 24 h. After that the cells were washed with PBS and then treated with 10 mM Rh 123 for 30 min. The fluorescence intensity was evaluated at the excitation wavelength of 488 nm and the emission wavelength of 510 nm with a fluorescence microplate reader. $^{**}p < 0.01$ compared with control. $^{\#}p < 0.05$ compared with the group treated by $A\beta_{25–35}$ alone. $^{\#\#}p < 0.01$ compared with the group treated by $A\beta_{25–35}$ alone

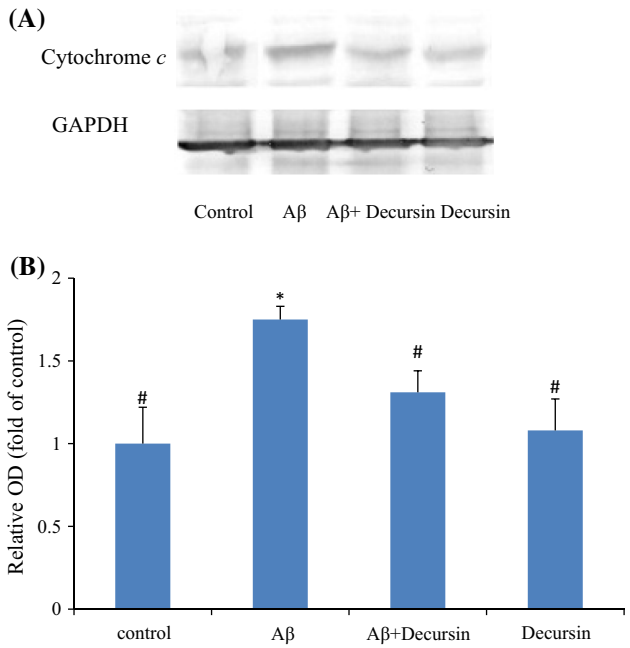

Fig. 7 Effect of decursin on the release of cytochrome *c* in $A\beta_{25–35}$-induced PC12 Cells. PC12 cells were pretreated with 10 μM decursin for 3 h and then incubated with and without 25 μM $A\beta_{25-35}$ for 24 h, after which the cytochrome *c* protein levels were determined by western blot analyses. Data are expressed as fold of control. $^{*}p < 0.05$ compared with control. $^{\#}p < 0.05$ compared with the group treated by $A\beta_{25–35}$ alone

Discussion

As a main component of senile plaques, Aβ is an important hallmark of Alzheimer's disease. The molecular mechanisms underlying Aβ-mediated neurotoxicity remain unclear. Recently, many in vitro and in vivo studies have shown that Aβ can directly induce neuronal death via the mechanism of apoptosis. Prevention of Aβ-triggered apoptosis might be one target to treat AD. Although many previous studies have shown that decursin exhibits potent neuroprotective activity, there has been no study on the effect of decursin on PC12 cells from apoptosis caused by Aβ. In this study, we investigate the protective effect and anti-apoptotic mechanism of decursin in $A\beta_{25–35}$-induced apoptosis in PC12 cells for the first time. Our present data illustrate that low doses of decursin (0.01–10 μM) exerts protective effects against $A\beta_{25–35}$-induced apoptosis in PC12 cells, as evidenced by the improved apoptotic rate (Fig. 3).

Mitochondria are currently regarded as playing a central role in Aβ toxicity therapy [29]. Mitochondrial dysfunction has been found in cells treated with Aβ [40], AD transgenic mice [10], platelets from AD patients [31], as well as postmortem brains of AD patients [8]. Mitochondrial dysfunction is consistent with intracellular ROS production and changes in MMP during apoptosis [20, 37]. In the present study, we found that $A\beta_{25–35}$ significantly increased MMP loss (Fig. 6) and induced the overproduction of intracellular ROS (Fig. 4). Pretreatment with decursin was able to restore the MMP and to decrease ROS production.

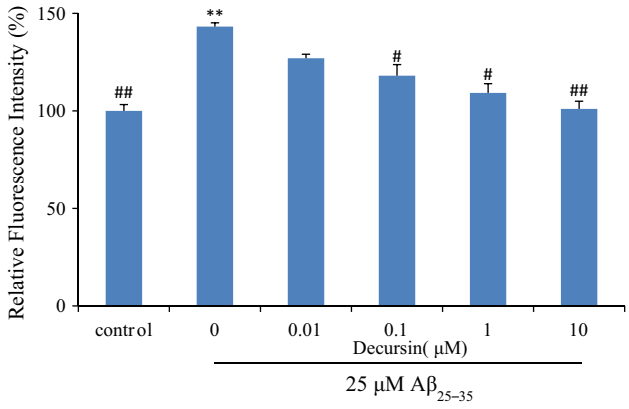

Fig. 8 Effect of decursin on caspase-3 activity in $A\beta_{25–35}$-induced PC12 Cells. PC12 cells were pretreated with various concentrations of decursin for 3 h and then incubated with and without 25 μM $A\beta_{25–35}$ for 24 h. The activity of caspase-3 was monitored spectrophotometrically at 405 nm. $^{**}p < 0.01$ compared with control. $^{\#}p < 0.05$ compared with the group treated by $A\beta_{25–35}$ alone. $^{\#\#}p < 0.01$ compared with the group treated by $A\beta_{25–35}$ alone

These results indicated that decursin prevents cell apoptosis by blocking activation of the mitochondrial apoptotic pathway.

Mitochondria pathway of apoptosis is always separated into two different pathways, simply known as caspase-dependent and caspase-independent pathways [18, 19, 36]. The mitochondrial pathway of apoptosis is regulated by the Bcl-2 family proteins consisting of several homologous proteins including anti-apoptotic proteins such as Bcl-2 and proapoptotic proteins including Bax [22]. Numerous studies have shown that Bcl-2, as a negative regulator of cell death, protects cells against apoptosis induced by various stimuli in a wide variety of cell types [21], whereas Bax is a positive regulator of cell death which promotes or accelerates cell death. Elevations in proapoptotic proteins, such as Bax, are believed to stimulate mitochondrial generation of ROS and contribute to nerve cell death in neurodegenerative diseases. Moreover, overexpression of Bcl-2 disrupts the proapoptotic proteins of Bax and prevents the mitochondrial release of cytochrome *c*, thereby inhibiting the activation of caspases and ultimately apoptosis [3, 7]. Thus, the balance of pro- and anti-apoptotic proteins is an important determinant for cell survival or death [25, 38]. In the present study, treated with $A\beta_{25-35}$ down-regulated the expression of anti-apoptotic Bcl-2 protein, and up-regulated the expression of proapoptotic Bax protein in PC12 cells, which were ameliorated in the presence of decursin pretreatment (Fig. 4). Cytochrome *c* is a mitochondrial protein, whose release into the cytosol is regulated by a protein belonging to the Bcl-2 family. Data showed that the release of cytochrome *c* from mitochondria into the cytosol was increased in $A\beta_{25-35}$-induced PC12 cells. And we observed a recovered level of cytosol cytochrome *c* in PC12 cells pretreated with decursin (Fig. 7). Apoptosis is actively regulated by several members of the caspase family, including caspase-3, which is the major effector caspase of apoptotic signals that catalyzes the cleavage of many cellular regulatory proteins [5, 30]. Once cytochrome *c* is released into the cytosol, it interacts with procaspase-9 after which it switches on caspase-3, leading to caspase-dependent apoptosis [4, 5]. In this study, increased caspase-3 activity was observed in $A\beta_{25-35}$-treated PC12 cells. Pretreatment PC12 cells with decursin could effectively attenuate these changes (Fig. 8). The findings of parallel increases in caspase-3 activity and decreased in Bcl-2/Bax expression ratio in $A\beta_{25-35}$-treated PC12 cells indicate the involvement of the mitochondrial pathway in triggering the $A\beta_{25-35}$-induced apoptosis. The increase in Bcl-2/Bax expression ratio and the inhibition of caspase-3 activity in $A\beta_{25-35}$-treated cells suggest decursin protects PC12 cells from $A\beta_{25-35}$-induced apoptosis by suppressing the mitochondrial caspase-dependent apoptotic pathway.

In conclusion, our results show that decursin exerts a protective effect against $A\beta_{25-35}$-induced neurotoxicity in PC12 cells, which was mediated through stabilizing mitochondrial function and reducing neuronal apoptosis. However, the complete molecular milieu that links all these events needs further investigation. These observations not only suggest the use of decursin for the treatment of various neurological diseases on a scientific foundation but also render decursin a promising naturally occurring chemical constituent worthy of further development into pharmaceutical therapy for AD.

Acknowledgments We gratefully acknowledge financial support of this work by Guangdong Medical College foundation (No.B2011011), Zhanjiang Science and Technology Planning Project (No. 2012C310 4018), Shenzhen Science and Technology Planning Project (No. 201 302173).

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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