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Original Article

Protective effects of compound FLZ, a novel synthetic analogue of squamosamide, on β-amyloid-induced rat brain mitochondrial dysfunction *in vitro*

Fang FANG[§], Geng-tao LIU*

Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Aim: The aim of the present study was to assess the effects of *N*-[2-(4-hydroxyphenyl)ethyl]-2-(2,5-dimethoxyphenyl)-3-(3-methoxy-4-hydroxyphenyl) acrylamide (compound FLZ), a novel synthetic analogue of squamosamide, on the dysfunction of rat brain mitochondria induced by $A\beta_{25-35}$ *in vitro*.
Methods: Isolated rat brain mitochondria were incubated with aged $A\beta_{25-35}$ for 30 min in the presence and absence of FLZ (1−100 μmol/L). The activities of key mitochondrial enzymes, the production of hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\cdot-}$), and the levels of glutathione (GSH) in mitochondria were examined. Mitochondrial swelling and the release of cytochrome *c* from mitochondria were assessed by biochemical and Western blot methods, respectively.
Results: Incubation of mitochondria with aged $A\beta_{25-35}$ inhibited the activities of α-ketoglutarate dehydrogenase (α-KGDH), pyruvate dehydrogenase (PDH) and respiratory chain complex IV. It also resulted in increased H_2O_2 and $O_2^{\cdot-}$ production, and decreased the GSH level in mitochondria. Furthermore, it induced mitochondrial swelling and cytochrome *c* release from the mitochondria. The addition of FLZ (100 μmol/L) prior to treatment with $A\beta_{25-35}$ significantly prevented these toxic effects of $A\beta_{25-35}$ on the mitochondria.
Conclusion: FLZ has a protective effect against $A\beta_{25-35}$-induced mitochondrial dysfunction *in vitro*.

Keywords: compound FLZ; β-amyloid; mitochondrial dysfunction; mitochondrial key enzymes; cytochrome *c*
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Introduction

Mitochondria play an important role in the regulation of cell survival and death. Many lines of evidence suggest that mitochondrial dysfunction is critical to the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative disorders[1–4]. Additionally, mitochondrial dysfunction has been proposed as a hallmark of β-amyloid (Aβ)-induced neuronal toxicity in the development and pathogenesis of AD[5–10]. Therefore, anti-AD pharmacological studies have focused intensively on the potential protective effects of stabilizing or restoring mitochondrial function as a therapy against AD[1, 11].

The natural product squamosamide was isolated from the leaves of *Annona squamosa*. Compound FLZ (*N*-[2-(4-hydroxyphenyl)ethyl]-2-(2,5-dimethoxyphenyl)-3-(3-methoxy-4-hydroxyphenyl) acrylamide) is a novel synthetic cyclic derivative of squamosamide with a molecular weight of 449.5 (Figure 1). Our previous studies demonstrated that compound FLZ protected against dopamine- and MPP^+-induced apoptosis in PC12 and SH-SY5Y cells. This protective effect is thought to be the result of inhibition of cytochrome *c* release from mitochondria and subsequent caspase-3 activation[12, 13]. In addition, our *in vivo* studies also showed that compound FLZ improved abnormal behaviors caused by the functional disturbance of dopaminergic and cholinergic neurons. Furthermore, this compound also improved the prognosis of mouse Parkinson's models (created through treatment with MPTP). Thus, FLZ possesses a strong neuroprotective property[12]. In addition, FLZ was also shown to

* Correspondence to Prof Geng-tao LIU.
E-mail liugt@imm.ac.cn/gtliu2002@yahoo.com
§ Now at *the Department of Pharmacology, School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100029, China*.
E-mail tyfangfang@eyou.com
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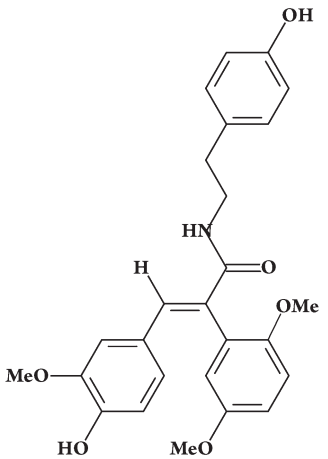

Figure 1. Chemical structure of FLZ.

reduce the impairment in learning and memory and the damage to the hippocampus induced by intracerebroventricular (icv) injection of $A\beta_{25-35}$ in mice. In SH-SY5Y cells, FLZ was also shown to inhibit apoptosis induced by $A\beta_{25-35}$[14, 15]. In a recent study, FLZ was effective in protecting against LPS- and MPTP-induced neurotoxicity in dopaminergic neurons and mice[16]. Based on these results, the present study examines whether FLZ can reduce $A\beta_{25-35}$-induced dysfunction of isolated rat brain mitochondria.

Materials and methods

Materials Compound FLZ was kindly provided by Prof Xiao-tian LIANG (Department of Pharmaceutical Chemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China). FLZ is a white powder of 99% purity and was first dissolved in absolute ethanol and then diluted with 0.9% saline to a final ethanol concentration of less than 0.5%. $A\beta_{25-35}$ (Sigma, St Louis, MO, USA) was dissolved in sterile double-distilled water at a concentration of 2 mg/mL, aged at 37 °C for 4 days, and subsequently stored at -20 °C until use. Aggregation of $A\beta_{25-35}$ was verified by the thioflavin-T fluorometric assay[17]. 5,5′-Dithio-*bis*(2-nitrobenzoic) acid (DTNB), SDS, thiamine pyrophosphate (TPP), *p*-iodonitrotetrazolium violet (INT), *D*,*L*-dothiothreitol (DTT), CoA, NAD and cytochrome *c* were purchased from Sigma (St Louis, MO, USA). Trichloroacetic acid (TCA), thiobarbituric acid (TBA) and other chemical reagents were of analytical grade and obtained from Beijing Chemical Factory (China).

Animals Male Wistar rats (220–250 g) were obtained from the Center of Experimental Animals, Chinese Academy of Medical Sciences (Grade II, Certificate No SCXK-Jing2004-0001). They were housed in groups of 5 or 6 and had access to laboratory food and water *ad libitum*. Rats were maintained in a thermo-regulated environment (23±1 °C, 50%±5% humidity) in a 12-h light/dark cycle. All animal experiments were conducted in accordance with the guidelines established by the Animal Care Committee of the Peking Union Medical College and the Chinese Academy of Medical Sciences.

Preparation of rat brain mitochondria Non-synaptic brain mitochondria were isolated from male Wistar rats using the published methods of Lai and Clark[18]. This preparation of nonsynaptic mitochondria is metabolically active, well coupled, and relatively free from non-mitochondrial contaminants[18, 19]. Mitochondrial protein concentration was determined by the Lowry method[20]. The purified mitochondria fraction was re-suspended in isolation medium (250 mmol/L sucrose, 0.5 mmol/L potassium EDTA, 10 mmol/L Tris-HCl, pH 7.4) at 4 °C. A mitochondrial protein concentration of 1 mg/mL was used for experiments.

Determination of mitochondria swelling Changes in mitochondrial swelling were monitored by tracking the changes in the apparent absorbance at 520 nm on a spectrophotometer over time[21]. The fresh mitochondrial protein was energized with 5 mmol/L succinate for 2 min prior to the experiments. The reaction in 1 mL of the incubation buffer (100 mmol/L sucrose,100 mmol/L KCl, 2 mmol/L KH_2PO_4, 10 μmol/L EGTA, 5 mmol/L HEPES, pH 7.4) containing 0.15 mg of mitochondria protein was initiated by continuous stirring at 30 °C. $A\beta_{25-35}$ was incubated with mitochondria for 5 min before the addition of succinate. Mitochondrial swelling was assayed by monitoring light scattering at 520 nm for 30 min on a spectrophotometer. Various concentrations of FLZ (1–100 μmol/L) were pre-incubated with mitochondria for 10 min before the addition of $A\beta_{25-35}$. The activity of α-ketoglutarate dehydrogenase (α-KGDH) was assayed spectrophotometrically at 25 °C by measuring the rate of increase in absorbance due to formation of NADH at 340 nm ($\varepsilon=6.23\times10^3$)[22]. The assay mixture contained 0.2 mmol/L thiamine pyrophosphate (TPP), 2 mmol/L NAD, 0.2 mmol/L CoA, 1 mmol/L $MgCl_2$, 0.3 mmol/L *D*,*L*-dithiothreitol (DTT), 0.1% (*v*/*v*) Triton X-100, 10 mmol/L α-ketoglutarate, 130 mmol/L HEPES-Tris pH 7.4, and mitochondrial suspension (0.1 mg protein/mL). The reaction was initiated by the addition of CoA and the initial rate was measured.

The activity of pyruvate dehydrogenase (PDH) was assayed using the published method[24]. Briefly, the initial buffer was made up of 50 mmol/L Tris-HCl, 0.5 mmol/L

EDTA, and 0.2% (*v*/*v*) Triton X-100 (pH 7.8), 2.5 mmol/L NAD, 0.1 mmol/L CoA, 1 mmol/L $MgCl_2$, 0.1 mmol/L oxalate, 1 mg of bovine serum albumin, 0.6 mmol/L INT, 6.5 mmol/L phenazine methosulfate, 0.2 mmol/L TPP, mitochondrial suspension (0.1 mg protein/mL) and 5 mmol/L pyruvate to a final volume of 1 mL. After addition of 4 mmol/L TPP (50 μL) and mitochondrial suspension (50 μL, 100 μg protein), the contents were mixed, and the tubes were placed in a water bath at 37 °C for 5 min. A stable baseline at 500 nm was then obtained for the reaction mixture in a double beam spectrophotometer. The reaction was started by adding pyruvate to the cuvette. The absorbance of the reaction mixture at 500 nm was recorded initially and then again after the reaction had proceeded for 2 min at 30 °C. Units of PDH activity were calculated using a molar extinction coefficient of 15.4×10^3 for the reduced dye.

The activity of complex IV was measured in a final volume of 1 mL of the reaction buffer (140 mmol/L KCl, 10 mmol/L HEPES, pH 7.4) containing mitochondria and dithionite-reduced cytochrome *c*. The decrease in absorbance of cytochrome *c* as it was oxidized by the enzyme was monitored at 550 nm. The molar extinction coefficient for cytochrome *c* of 19.6×10^3 was used[25].

Determination of H_2O_2 and $O_2^{\cdot-}$ generation and of GSH content in mitochondria H_2O_2, $O_2^{\cdot-}$, and GSH content were measured using a spectrophotometer. Mitochondrial protein (0.5 mg/mL) was incubated with different concentrations of $A\beta_{25-35}$ in the reaction buffer (140 mmol/L NaCl, 5.5 mmol/L glucose, 10 mmol/L potassium phosphate, pH 7.4) containing 50 μg/mL horseradish peroxidase and 100 μg/mL phenol red at 37 °C for 30 min. FLZ (1–100 μmol/L) was incubated with the mitochondria for 10 min at 37 °C before the addition of $A\beta_{25-35}$. The incubation was stopped by adding 10 μL 1 mol/L NaOH and then centrifuged at 3000 r/min for 10 min. The supernatant was removed. The absorbance was measured at 595 nm with 655 nm as the reference wavelength. H_2O_2 production was calculated using a standard curve of H_2O_2[26].

$O_2^{\cdot-}$ content was measured by NBT oxidization. Mitochondrial protein (0.5 mg/mL), was incubated with different concentrations of $A\beta_{25-35}$ and 20 μL 1% NBT in the reaction buffer (140 mmol/L NaCl, 5.5 mmol/L glucose, 10 mmol/L potassium phosphate, pH 7.4) at 37 °C for 30 min. Various concentrations of FLZ (1–100 μmol/L) were incubated with the mitochondria for 10 min at 37 °C before addition of $A\beta_{25-35}$. The reaction was terminated in an ice bath and centrifuged at 3000 r/min for 10 min. The supernatant was removed, and the pellet was dissolved by addition of DMSO. The absorbency was measured at 570 nm[27]. GSH content was measured by the DTNB method. Mitochondrial suspension was incubated as described above for the mitochondrial swelling measurements. After incubation, mitochondria were isolated *via* centrifugation at 12 000×*g* for 10 min at 4 °C. The supernatant was removed, and the protein was precipitated from the mitochondrial pellet by the addition of 20% TCA with 0.1 mol/L PBS, followed by centrifugation at 3000 r/min for 10 min. The supernatant, containing GSH from inside the mitochondria, was removed and measured using the DTNB method[28].

Determination of cytochrome *c* content in mitochondria by Western blot The preparation of mitochondria was the same as for the mitochondrial swelling experiments. At the end of the 30 min incubation, each mitochondrial suspension was rapidly centrifuged at 15 000 r/min for 5 min. The resulting pellets were resuspended in 200 μL RIPA (25 mmol/L Tris-HCl, 150 mmol/L NaCl, 5 mmol/L EDTA, 5 mmol/L EGTA, 1 mmol/L PMSF, 1% Triton X-100, 0.5% Nonidet P40, 10 mg/L aprotinin and 10 mg/L leupeptin) and placed on ice for 30 min. They were then centrifuged at 12 000×*g* for 15 min and the supernatant was removed and stored at -70°C. Protein concentration was determined following standard protocols[20].

The protein samples (100 μg) were mixed with the buffer (100 mmol/L Tris-HCl, pH=6.8, 200 mmol/L DTT, 4% SDS, 0.2% bromophenol blue and 20% glycerol). Proteins were separated on 15% acrylamide gels after denaturation at 100 °C for 5 min and then transferred to PVDF membranes. The membranes were incubated for 2 h at room temperature (RT) in blocking buffer (25 mmol/L Tris-HCl, pH=7.6, 150 mmol/L NaCl, and 0.01% Tween-20) containing 5% fat-free milk. Then, the blots were incubated with a primary monoclonal antibody to cytochrome *c* (dilution of 1:1000 in blocking buffer containing 5% fat-free milk) for 3 h at RT with gentle shaking. After being washed three times with blocking buffer (5 min each), the immunoblots were incubated for 1 h at RT with anti-mouse antibody-alkaline phosphatase (1:500 dilution in blocking buffer).

This was followed by three washes with blocking buffer (5 min each). The blot was then visualized with NCBI/NBT. Finally, the blot was scanned and its density was analyzed using the software[21].

Statistical analysis Data in the mitochondrial swelling experiments are expressed as the mean±SD; other data are expressed as the mean±SEM. Statistical significance was determined using one-way ANOVA, and *P* values lower than 0.05 were considered statistically significant.

Results

Effect of FLZ on $A\beta_{25-35}$-induced mitochondrial swelling Usually, the opening of the mitochondrial permeability transition pore (PTP) is monitored by following the decrease in absorbance associated with mitochondrial swelling. The addition of various doses of $A\beta_{25-35}$ (10 μmol/L to 30 μmol/L) caused a decrease in the absorbance in a concentration-dependent manner, which indicated mitochondrial swelling. Based on these results, we selected 20 μmol/L $A\beta_{25-35}$ as an appropriate concentration for observing the effect of FLZ on mitochondrial swelling. The results showed that 10 and 100 μmol/L FLZ significantly counteracted the decrease in absorbance induced by $A\beta_{25-35}$ (Figure 2). FLZ 1 μmol/L had no effect.

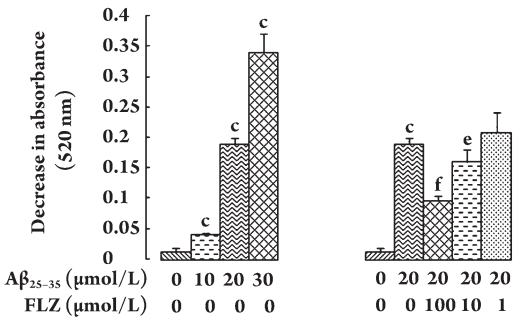

Figure 2. Quantification of absorbance changes was calculated by the decrease in the absorbance at 30 min compared with the absorbance at 0 min. Mitochondria were treated with $A\beta_{25-35}$ for 30 min. FLZ (1–100 μmol/L) was pre-incubated with mitochondria for 10 min before the addition of $A\beta_{25-35}$. n=3. Values are expressed as mean±SD. ^cP<0.01 *vs* control (without $A\beta_{25-35}$ and FLZ). ^eP<0.05, ^fP<0.01 *vs* $A\beta_{25-35}$ (20 μmol/L) group.

Effect of compound FLZ on key enzymes of mitochondria intoxicated with $A\beta_{25-35}$ $A\beta_{25-35}$ caused a dose-dependent reduction in the activities of complex IV, α-KGDH and PDH in isolated rat brain mitochondria. The susceptibility of these enzymes to the treatment with $A\beta_{25-35}$ was different. To induce significant inhibition of complex IV and PDH activities, 80–100 μmol/L $A\beta_{25-35}$ was necessary, whereas $A\beta_{25-35}$ 10 μmol/L was enough to inhibit the activity of α-KGDH (Figure 3).

Based on these results, 100 μmol/L $A\beta_{25-35}$ was used to study the effect of compound FLZ on complex IV and PDH activities, and 10 μmol/L $A\beta_{25-35}$ was used to test α-KGDH activity. The results showed that the addition of 100 μmol/L FLZ significantly prevented the $A\beta_{25-35}$-induced reduction in the activities of these three key mitochondrial enzymes

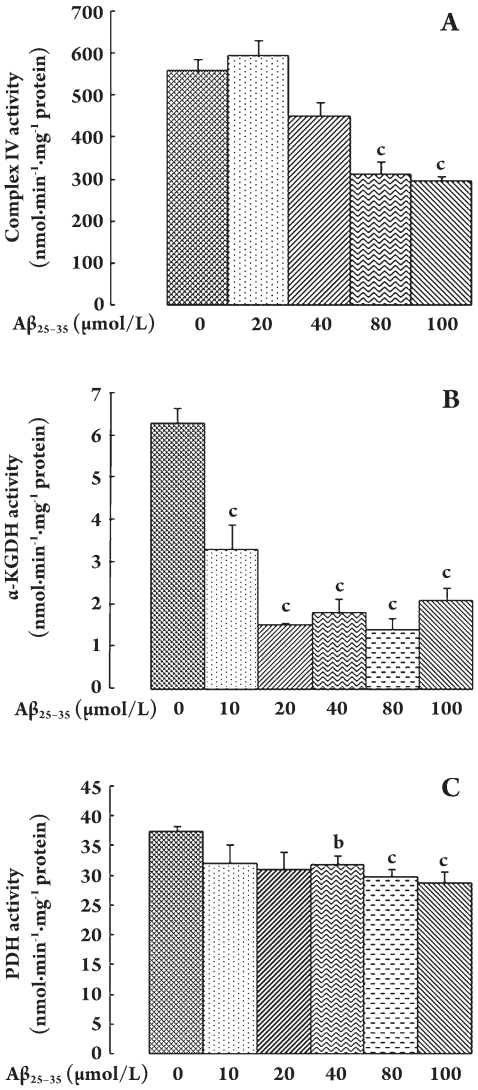

Figure 3. Effect of $A\beta_{25-35}$ on complex IV, α-KGDH and PDH activities of rat brain mitochondria *in vitro*. (A) Complex IV activity; (B) α-KGDH activity; (C) PDH activity. The isolated rat brain mitochondria (100 μg/mL) were incubated with various concentrations of $A\beta_{25-35}$ at 37 °C for 30 min, the three enzyme activities were determined with different methods as described in Material and Methods. n=3–5. Data are expressed as mean±SEM. ^bP<0.05, ^cP<0.01 *vs* control (without $A\beta_{25-35}$).

(Figure 4).

Effect of FLZ on $A\beta_{25-35}$-induced changes in H_2O_2, $O_2^{\cdot-}$ production and in GSH mitochondrial content $A\beta_{25-35}$ increased H_2O_2 and $O_2^{\cdot-}$ production in the isolated rat brain mitochondria in a dose dependent manner (Figure 5). The effects of 50 and 100 μmol/L $A\beta_{25-35}$ were statistically significant. $A\beta_{25-35}$ 50 μmol/L also decreased GSH content in the mitochondria (Figure 7). FLZ (100 μmol/L)

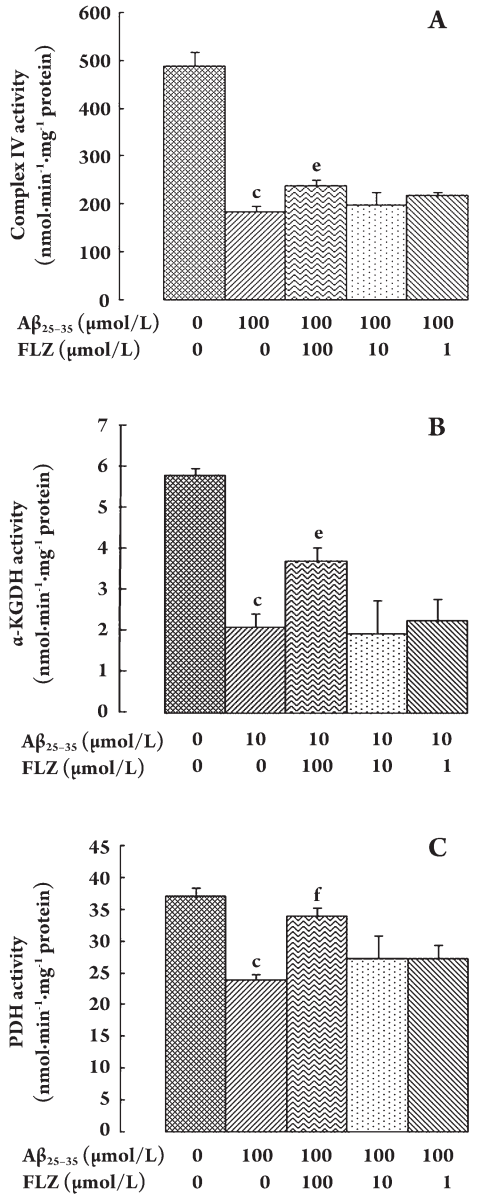

Figure 4. Effect of FLZ on complex IV, α-KGDH, and PDH activity of rat brain mitochondria inhibited by $A\beta_{25-35}$ *in vitro*. (A) Complex IV activity; (B) α-KGDH activity; (C) PDH activity. Isolated rat brain mitochondria (100 μg/mL) were incubated with the indicated concentrations of $A\beta_{25-35}$ at 37 °C for 30 min. FLZ (1–100 μmol/L) was pre-incubated with mitochondria for 10 min before the addition of $A\beta_{25-35}$. n=3. Values are expressed as mean±SEM. ^{c}P<0.01 *vs* control (without $A\beta_{25-35}$ and FLZ); ^{e}P<0.05, ^{f}P<0.01 *vs* $A\beta_{25-35}$ treated group.

significantly protected against the $A\beta_{25-35}$-induced increase in H_2O_2 and $O_2^{\cdot-}$ production and the decrease of GSH content in mitochondria (50 μmol/L $A\beta_{25-35}$) (Figure 6, 7).

Effect of FLZ on $A\beta_{25-35}$-induced reduction in cytochrome *c* content in mitochondria $A\beta_{25-35}$ 50 μmol/L induced a significant reduction in cytochrome *c* content in mitochondria. FLZ 100 μmol/L markedly prevented the release of cytochrome *c* from the mitochondria (Figure 8).

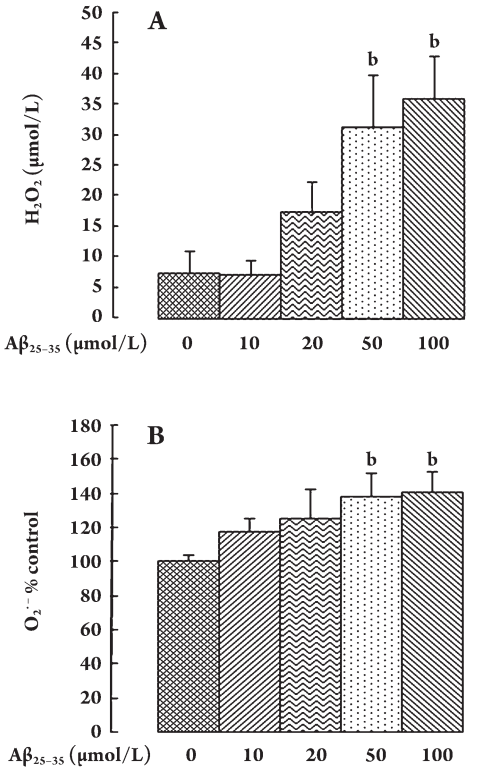

Figure 5. Effect of $A\beta_{25-35}$ on H_2O_2 and $O_2^{\cdot-}$ production in isolated rat brain mitochondria *in vitro*. (A) H_2O_2 production. (B) $O_2^{\cdot-}$ production. The isolated rat brain mitochondria were incubated with different concentrations of $A\beta_{25-35}$ for 30 min. n=4. mean±SEM. ^{b}P<0.05 *vs* control (without $A\beta_{25-35}$).

Discussion

Mitochondria are essential for neuronal function and morphological alterations of the mitochondria have been found in AD patients[29, 30]. Postmortem analysis of AD brains shows significant disturbances in mitochondrial energy and glucose metabolism[31, 32]. Impairment of mitochondrial oxidative phosphorylation, together with a reduction in the activities of cytochrome *c* oxidase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, seem to be responsible for the decrease in glucose metabolism and energy production found in AD brains[3, 33, 34]. Numerous studies have shown that mitochondrial function can be disturbed by increasing secretion and intracellular accumulation of Aβ or by exposure to extracellular Aβ[6, 7, 8, 23, 35, 36]. Additionally, some studies have also shown that Aβ accu-

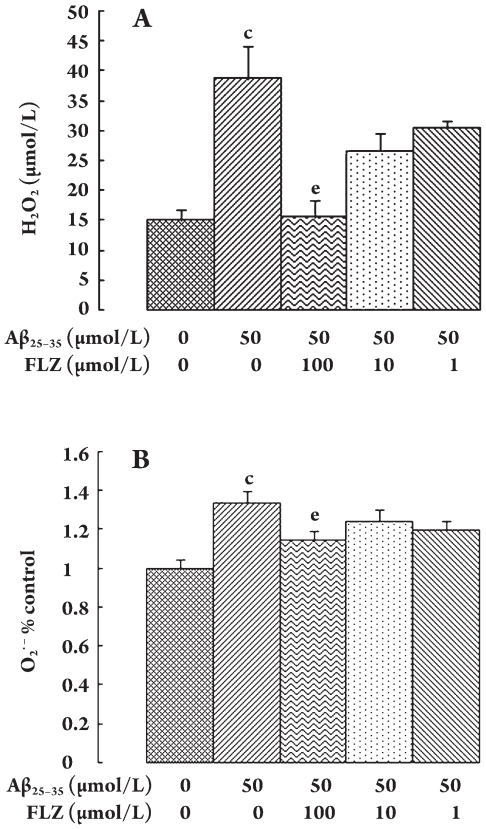

Figure 6. Effect of FLZ on H_2O_2 and $O_2^{\cdot-}$ production in isolated rat brain mitochondria induced by $Aβ_{25-35}$ *in vitro*. (A) H_2O_2 production. (B) O2$^{\cdot-}$ production. Isolated rat brain mitochondria (500 μg protein/mL) were incubated with 50 μmol/L $Aβ_{25-35}$ at 37 °C for 30 mim, FLZ (1–100 μmol/L) was pre-incubated with mitochondria for 10 min before the addition of $Aβ_{25-35}$. n=4. Values are expressed as mean±SEM. ^cP<0.01 *vs* control group (without $Aβ_{25-35}$ and FLZ); ^eP<0.05 *vs* $Aβ_{25-35}$ treated group.

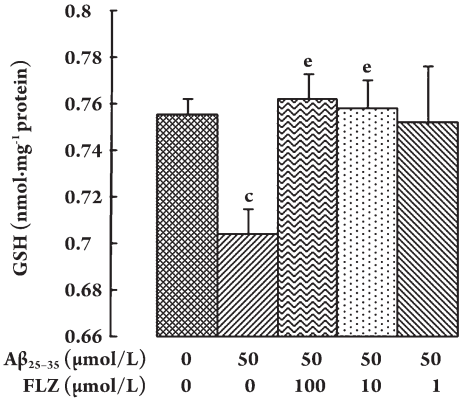

Figure 7. Effect of FLZ on decrease of GSH content induced by $Aβ_{25-35}$ in rat brain mitochondria. $Aβ_{25-35}$ was incubated with rat brain mitochondria for 30 min, and various concentrations of FLZ (1–100 μmol/L) were incubated with mitochondria for 10 min before the addition of 50 μmol/L $Aβ_{25-35}$. n=3. Mean±SEM. ^cP<0.01 *vs* control (without $Aβ_{25-35}$ and FLZ); ^eP<0.05 *vs* $Aβ_{25-35}$ treated group.

mulates within the neuronal mitochondria in brains of AD patients[10, 37, 38]. Thus, there is evidence for a functional link between mitochondrial dysfunction and the pathogenesis of AD.

The main pathway for glucose oxidation in the brain is the tricarboxylic acid (TCA) cycle, which takes place in the mitochondria. The α-ketoglutarate dehydrogenase and pyruvate dehydrogenase are two key enzymes of the limiting step in the TCA cycle. In addition, pyruvate dehydrogenase provides acetyl CoA for the synthesis of acetylcholine. α-Ketoglutarate, the substrate of α-ketoglutarate dehydrogenase, is generated by glutamate dehydrogenase during the oxidative deamination of glutamate. Glutamate is an excitatory neurotransmitter that, in excess, can lead to neuropathology. A reduction in the activities of these two enzymes could lead to a decrease in energy production, the accumulation of glutamate, and a reduction in acetylcholine synthesis. Acetylcholine and glutamate have been linked to the impairment in learning and memory observed in AD patients[39, 40]. The complex IV activity is a rate-limiting step of oxidative phosphorylation (OXPHOS) in mitochondria. The decrease in complex IV activity could result in the generation of reactive oxygen species (ROS) by reducing energetic synthesis and arresting the electronic transmission in the mitochondrial respiratory chain (MRC). Mitochondria are the largest source of ROS, and they also are their targets. If ROS is generated, it can damage mitochondrial proteins and membranes and also cause an increase in the number of mutations in the mtDNA. This inhibits the electron transport through the complexes, further stimulating mitochondrial ROS production. This results in a vicious cycle in cells[41].

In the present study, $Aβ_{25-35}$ was shown to inhibit the activities of three key enzymes in isolated rat brain mitochondria, to increase H_2O_2 and $O_2^{\cdot-}$ production, and to decrease GSH content in the mitochondria. These results confirmed that mitochondrial dysfunction and oxidative stress contributed to Aβ toxicity. Pretreatment with FLZ significantly increased the activities of α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and complex IV. And FLZ also decreased H_2O_2 and $O_2^{\cdot-}$ production while also increasing GSH content in mitochondria. All these results indicate that FLZ protected against $Aβ_{25-35}$-induced dysfunction and oxidative stress in rat brain mitochondria. Two possible mechanisms have been proposed to explain how Aβ inhib-

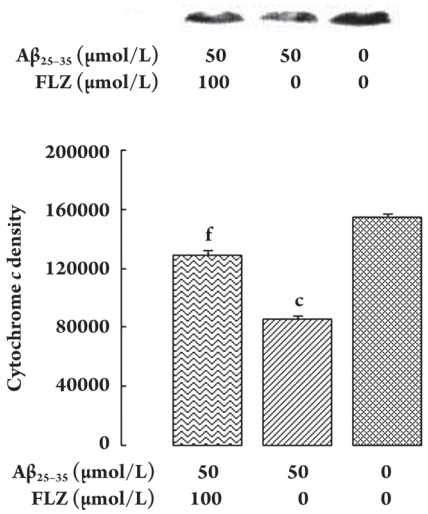

Figure 8. Effect of FLZ on the decrease in cytochrome *c* content in the rat brain mitochondria induced by $A\beta_{25-35}$. The rat brain mitochondria was previously incubated with 100 μmol/L FLZ for 10 min, and then incubated with 50 μmol/L $A\beta_{25-35}$ for 30 min. The cytochrome *c* was determined by Western blot method as described in the Materials and methods. n=3. Values are expressed as mean±SEM. $^{c}P < 0.01$ *vs* control (without $A\beta_{25-35}$ and FLZ); $^{f}P<0.01$ *vs* $A\beta_{25-35}$ treated group.

its mitochondrial enzymes[6]. The first one is based on the direct interaction between Aβ with these enzyme complexes in mitochondria, whereas the second one involves the production of reactive oxygen species that, in turn, damage protein subunits and/or essential cofactors of mitochondria. We had previously shown that FLZ inhibited microsomal lipid peroxidation induced by Fe^{2+}-cysteine. Furthermore, FLZ also inhibited the production of superoxide induced by LPS, which is probably mediated through inhibition of NADPH oxidase activity[16, 42]. The mechanism by which FLZ protects against the damaging effect of $A\beta_{25-35}$ on mitochondria might be related to its antioxidant property or to competing for the binding site with the Aβ.

Furthermore, compound FLZ was shown to inhibit the $A\beta_{25-35}$-induced mitochondrial swelling and cytochrome *c* release from mitochondria. The opening of mitochondrial permeability transition pores (PTP) is indicated by mitochondrial swelling, which is expressed as a decrease in the optical density of a mitochondrial suspension. The level of mitochondrial function is related to the regulation of PTP opening. Some anti-AD agents that prevent pathologic PTP opening may preserve mitochondrial function. Aβ-induced mitochondrial dysfunction is also mediated by the opening of mitochondrial PTP[43, 44]. This mitochondrial PTP opening may promote cytochrome *c* release and cause apoptosis or necrosis of cells. Cytochrome *c* is an essential component of the mitochondrial respiratory chain and can shuttle electrons between respiratory chain complexes III and IV because of its heme group. The release of cytochrome *c* leads to two potentially lethal consequences: the decrease in ATP synthesis and the overproduction of the radical superoxide anion[43]. In any case, the suppression of lipid peroxidation and inhibition of PTP opening by FLZ contribute to preserving mitochondrial function.

In brief, compound FLZ has protective effects on the brain mitochondrial dysfunction induced by $A\beta_{25-35}$. The present results provide strong evidence to further support FLZ as a neuroprotective agent.

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Author contribution

Geng-tao LIU designed research and revised the paper; Fang FANG performed research and wrote the paper .

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