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Pharmacological Study of the Novel Compound FLZ Against Experimental Parkinson's Models and Its Active Mechanism

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Abstract

FLZ is a synthetic new derivative of squamosamide. Pharmacological study found that FLZ given orally improved the abnormal behavior caused by the functional disturbance of dopaminergic and cholinergic neurons in mice. FLZ significantly increased the content of dopamine and its metabolites in striatum in MPTP model mice. FLZ also remarkably protected dopaminergic PC-12 cells against dopamine and MPP^+ induced injury and apoptosis in vitro. The compound inhibited the formation of dopamine–melanin and protein polymers. Additionally, FLZ inhibited cytochrome-*c* release from mitochondria and caspase-3 activation by dopamine in PC-12 cells. The above results suggest that compound FLZ possesses anti-PD activity through neuroprotection.

Index Entries: Parkinson's disease; dopaminergic neurons; oxidative stress; apoptosis; compound FLZ; MPTP; dopamine.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder caused by the disturbance of the extrapyramid system's function. The characteristic pathogenesis of PD is degeneration of dopaminergic neurons in the nigra zona compacta and decrease of the neuron-transmitter dopamine content in the striatum, which lead to dysfunction of the dopaminergic neurons and relative domination of cholinergic function *(1)*. The incidence of PD is about 10% in people over 65 yr of age. There are an estimated 1 million PD patients in the United States and tens of millions PD patients worldwide *(2)*. Although the etiology of PD is still unknown, genetic factors, infections,

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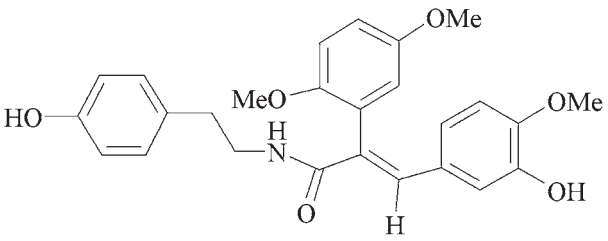

Fig. 1. Chemical structure of FLZ.

immunological abnormalities, aging, oxidative stress, and toxins (endogenous and exogenous) might all be involved in the development of PD *(3–9)*. Among these factors, oxidative stress and impaired mitochondrial function have been considered to play important roles in PD development *(10,11)*.

Pharmacological therapy of PD has made significant advances and improvements especially over the last 10 yr. A number of new treatments and new strategies have emerged. However, drugs currently used for PD therapy only improve clinical symptoms but hardly retard the progression of PD. Moreover, side effects are often observed in the long-term treatment of PD. New drugs with good curative effect and fewer side effects are urgently needed for PD therapy. Recently, attention has been focused on a neuroprotective strategy for pharmacotherapy of PD *(2,12–15)*.

FLZ is a synthetic new derivative of squamosamide (structure shown in Fig. 1). Our previous study demonstrated that FLZ has a strong action to protect against damage and apoptosis of primary cultured rat brain neurons exposed to hydrogen peroxide, glutamate, *N*-methyl-D-asparatate (NMDA), hemoglobin, and ischemia–reoxygenation *(16)*, indicating that FLZ might possess a neuroprotection property. As a result, we want to study further whether FLZ has action to improve animal abnormal behavior of the Parkinson's model and protect against neuron apoptosis. The main results of our study on this problem are briefly reviewed here.

Improvement Effect of FLZ on Abnormal Behavior in MPTP, Arecoline, Oxytremorine-Poisoned Mice and in 6-OH-DOPA-Injected Rats

Effect on MPTP Model

1-Methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) is a neuron toxin and used to produce a PD model *(17)*. Adult male C57/BL mice were tested for the ability to climb down a steel rod; only those mice that performed well were used and divided into groups of normal control, MPTP control, FLZ 37.5, 75, and 150 mg/kg treated groups, and L-DOPA 75 mg/kg treated group, respectively. All mice except the normal control group, received two doses of 50 mg/kg MPTP interperitoneally at an interval of 16 h. FLZ and L-DOPA were administered orally 40 min before each injection of MPTP and continued once daily for six doses after the second injection of MPTP. The climbing behavior of all mice was observed daily for 7 d and given different marks according to the degree of disturbance of climbing behavior. As a result, the MPTP control group mice developed disturbance of climbing behavior on the steel pole and did not recover until the d 7. The FLZ treatment (150 and 75 mg/kg) significantly accelerated the restoration of the abnormal behavior of mice in a dose-dependent manner. The efficacy of 75 mg/kg FLZ in improving the disturbance of climbing behavior corresponded to that of 75 mg/kg L-DOPA. The 37.5-mg/kg FLZ dose was ineffective.

In order to confirm whether the improving effect of FLZ on climbing behavior disturbance in the MPTP mouse model is specific, the effect of FLZ on alcohol-induced disturbance of climbing behavior was observed in mice with the same method as in the above MPTP experiment. A dose of 25% ethanol 10 mL/kg was administered orally to mice. The 150-mg/kg FLZ dose, which showed improvement of behavior disturbance in the MPTP experiment, was given orally

1 h before administration of 25% alcohol 10 mL/kg. FLZ showed no improving effect on disturbance of climbing ability in mice after ingestion of alcohol, indicating that the improving effect of FLZ on disturbance of pole climbing behavior in the experiment of MPTP is specific.

Effect on Arecoline- and Oxytremorine-Induced Trembling in Mice

For observing the effect of FLZ on trembling behavior, adult male Kunming strain mice were injected with arecoline and oxytremorin in the tail vein. The ED_{50} of trembling responses were measured by an up-down method. A dose of 150, 75, or 37.5 mg/kg FLZ or 75 mg/kg L-DOPA were administered orally, respectively, 1 h before injection of arecoline and oxytremorine. As a result, 150 and 75 mg/kg FLZ increased the trembling ED_{50} value of arecoline from 564.84 μg/kg of the control to 753.05 μg/kg and 599.7 μg/kg, respectively. The differences between control and FLZ-treated groups were significant ($p < 0.05$), calculated according to Dixon and Mood's method. The duration of trembling of the 150- and 75-mg/kg FLZ-treated group of mice was also shortened from 81.6 s of the control group to 63.6 s and 66.5 s, respectively. The 75-mg/kg L-DOPA dose was ineffective.

In the oxytremorine experiment, FLZ at doses of 150 and 75 mg/kg also increased the trembling ED50 value of oxytremorine from 51.0 μg/kg of the control to 93.0 μg/kg and 73.5 μg/kg ($p < 0.05$), respectively. The onset time (latency) of trembling development was postponed and the duration of trembling was shortened by the FLZ treatment. The 75-mg/kg l-DOPA dose was without such effect.

All the above results suggested that FLZ selectively improved the abnormal behavior caused by the functional imbalance of dopaminergic and cholinergic neurons in mice.

Effect of FLZ on Contralateral Turning Behavior in 6-OH DOPA Model Rats

6-OH–DOPA was injected unilaterally into rat striatum to damage dopaminenergic neurons *(18)*. Fourteen days later, a dose of 0.1 mg/kg apomorphine was injected to initiate cycling response. Positive cycle rotation rates were divided into three groups randomly and were treated with 150 mg/kg FLZ, 150 mg/kg, L-DOPA, or the vehicle (0.5% Na-CMC) once daily for 7 d. The rotation behavior of each rat was examined for 30 min on d 5 and d 7. It was found that all 11 control rats still showed rotation response within 7 d after each injection of apomorphine, whereas the number of rotation responses of FLZ-treated 12 rats decreased at d 5 and d 7. Similarly, the rotation number of six L-DOPA-treated rats was decreased.

Effect of FLZ on the Level of Dopamine and Its Metabolites and MDA Formation in MPTP Model Mice

Reduced level of dopamine and its metabolites in striatum is the characteristically biochemical changes in PD. The MPTP model was reproduced in mice and they were treated with FLZ with the same protocol as described in the above MPTP experiment. The death of mice was recorded 24 h after injection of MPTP and the survived mice were sacrificed on d 7 after the second injection of MPTP; the content of dopamine (DA) and its metabolites (DOPAC and HVA) in the striatum was determined by the high-performance liquid chromatography–electrochemical detection method *(19)*. Malondialdehyde (MDA) was determined by the thiobarbutric acid method *(20)*. In summary, MPTP induced about 50% of mouse death and a significant decrease of DA, DOPAC, and HVA levels in the striatum. Oral administration of FLZ (150 mg/kg, 75 mg/kg) significantly reduced the mortality of mice and markedly increased the content of DA and its metabolites DOPAC and HVA levels in the striatum of MPTP poisoned mice.

Malondialdehyde (MDA) is an end product of lipid peroxidation of biomembranes; the

cause of MPTP-induced neuron damage is the result of the generation of free radicals, which initiated lipid peroxidation of neuron cell membranes. The result of MDA determination indicated that MPTP injection induced a significant increase of MDA level in the striatum. The treatment of MPTP-poisoned mice with FLZ (150 mg/kg orally) remarkably reduced the MDA level in the striatum in comparison with the MPTP control group, whereas both FLZ and MPTP showed no effect on MDA level in the brain cortex of the mice.

Effect of FLZ on the Activity of Monooxidase B and Acetyl Cholinesterase of Rat Brain Monoamine In Vitro

L-DOPA is metabolized to DA by oxidase B (MAO-B). In order to study the effect of FLZ on the metabolic conversion of L-DOPA, FLZ (1×10^{-4}–1×10^{-6} mol/L) was preincubated with rat whole-brain homogenate at 37°C for 10 min, 1×10^{-5} mol/L MPTP was then added and incubated for another 30 min. MAO-B activity was then determined *(21)*. MPTP significantly reduced MAO-B activity. The addition of FLZ markedly counteracted the inhibition of MAO-B activity by MPTP ($p < 0.001$). The same concentrations of FLZ showed no effect on rat brain MAO-B activity in the absent of MPTP.

With the same incubation protocol, the effect of FLZ on acetyl cholinesterase (AchE) activity was examined *(22)*. As a result, the same concentrations of FLZ showed no effect on AchE activity. It is known that MPTP becomes toxic to the striatum by its conversion to its active form MPP^+ through brain MAO-B. It appears that the anti-parkinsonism effect of FLZ might be the result of the decrease of free radical generation and active metabolite MPP^+ formation of MPTP through inhibition of MAO-B activity.

Protective Effect of FLZ on Dopamine- and MPP^+-Induced Injury in Dopaminergic PC-12 Cell Line

The PC-12 cells are regarded as dopaminergic cells because it can generate and secrete dopaminergic neurotransmitter. The protective effect of FLZ on dopamine- and MPP^+-induced neural cell damage was investigated in PC-12 cells by the MTT method *(23)*. Both dopamine and MPP^+ decreased cell viability in time- and dose-dependent manners. The morphological injury such as round and semiadhesion of PC-12 cells was observed after incubation with DA or MPP^+. Dopamine, 5×10^{-4} mol/L, or MPP^+, 2×10^{-3} mol/L, cultured with PC-12 cells for 24 h resulted in the increase of DNA fragmentation *(24)*. Similar results were obtained in single-cell comet electrophoresis. When 5×10^{-5} mol/L FLZ was cultured with PC-12 cells for 30 min before the addition of DA or MPP^+, all of the above damages of PC-12 cells were notably reduced. After 5×10^{-4} mol/L DA or 2×10^{-3} mol/L MPP^+ incubation for 24 h, condensed DNA chromatin was observed under laser scan confocal microscope (LSCM) and transmission electron microscope. Cell apoptosis was determined after the cells cultured with 5×10^{-4} mol/L DA and 2×10^{-3} mol/L MPP^+ by flow cytometer analysis (FCA), which showed sub-G_1 peak, and by agarose gel electrophoresis (DNA ladder), which showed typical internucleosomal DNA degradation. The apoptosis of PC-12 cells was inhibited by preincubation of 5×10^{-5} mol/L FLZ with PC-12 cells for 60 min before the addition of dopamine or MPP^+.

Effect of FLZ on the Formation of High-Molecular-Weight Protein Polymers and Dopamine–Melanin in PC-12 Cells

The formation of protein polymers and melanin induced by DA has been considered to

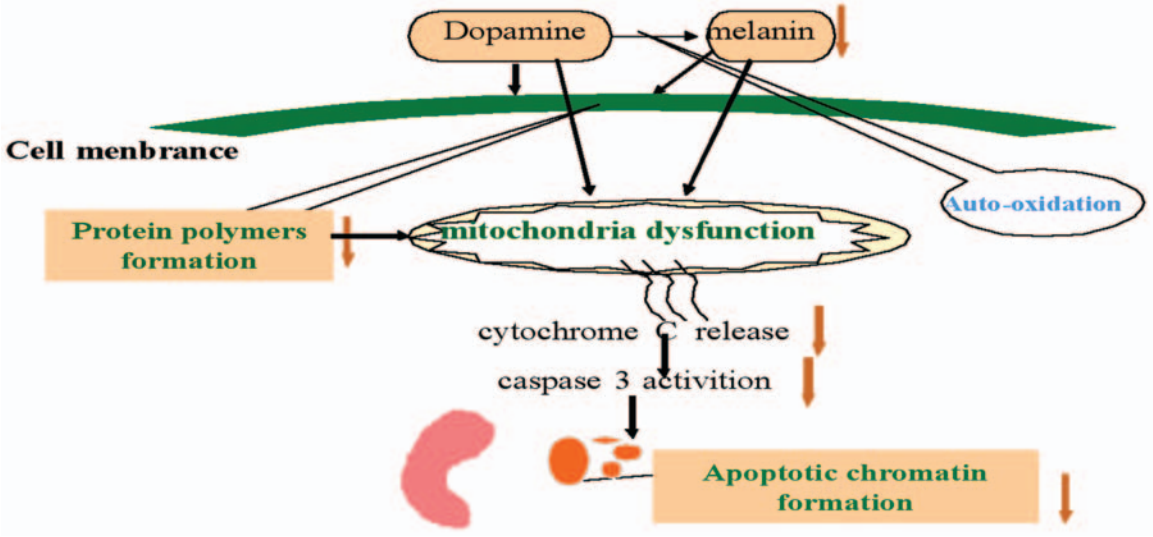

Fig. 2. Illustration of the mechanisms of FLZ against DA-induced neuro-cell apoptosis damage. ↓ represents inhibition by FLZ.

play an important role in PD pathogenesis. Thus, the effect of FLZ on DA-induced formation of protein polymers and melanin was examined with related methods *(25,26).* When 5×10^{-4} mol/L DA was cultured with PC-12 cells for 24 h in the presence of 5×10^{-5} mol/L, Fe^{2+} the formation of high-molecular-weight protein polymers was detected by sodium doclecyl sulfate–polyacrylamide gel electrophoresis. The 5×10^{-5} mol/L FLZ added 30 min before DA and Fe^{2+} addition markedly reduced the formation of high-molecular-weight protein polymers. Dopamine–melanin formation in PC-12 cells was enhanced with culture time prolonged and increased doses of dopamine. The 5×10^{-6}–5×10^{-5} mol/L FLZ preincubated with PC-12 cells for 30 min significantly decreased dopamine–melanin formation induced by 1×10^{-4} mol/L DA. FLZ also showed inhibitory effect on dopamine–melanin formation catalyzed by Cu^{2+} in vitro in a dose-dependent manner. All of the above results suggested that FLZ protected cells from toxin injury by inhibiting formation of dopamine–melanin and protein polymers.

Effect of FLZ on Release of Cytochrome-*c* From Mitochondria and Activation of Caspase-3 Induced by Dopamine in PC-12 Cells

Signal transduction of cytochrome-*c* and caspase-3 are involved in cell apoptosis. Dopamine incubated with PC-12 cells induced release of cytochrome-*c* from mitochondria and activation of caspase-3. The addition of 50 μmol/L FLZ to the incubation system markedly decreased the release of cytochrome-*c* and activation of caspase-3 induced by DA, suggesting that the anti-apoptosis effect of FLZ is through inhibition of cytochrome-*c* release and caspase-3 activation, as shown in Fig. 2.

In summary, FLZ possesses anti-PD action through neuroprotection. It is worthwhile to

translate FLZ from animal models to Parkinsonian patients.

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