Research report

Risperidone ameliorated Aβ1-42-induced cognitive and hippocampal synaptic impairments in mice

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**HIGHLIGHTS**

- Risperidone improved the cognitive impairment of AD mice.
- Risperidone inhibited the generation of Aβ and p-Tau.
- Risperidone inhibited the neurons apoptosis induced by Aβ1-42.

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**ABSTRACT**

Alzheimer's disease (AD) is a complex neurodegenerative disorder with cognitive impairment and major neuropathologic hallmark of amyloid-beta (Aβ) peptides. Risperidone, an atypical antipsychotic, can improve concentration and cognitive deficit in schizophrenia patients. In this study, behavior tests including Morris Water Maze test, Step-through passive avoidance test, Open Field test, Step-Down test, Hole-Board test and Novel object recognition test were preformed to examine the effect of Risperidone on Aβ1-42-induced cognitive dysfunction in both long-term and short-term memory. Furthermore, ELISA assay was conducted to measure the levels of Aβ1-42, BACE1 and p-Tau in the hippocampus and cortex. Moreover, primary cortical neuron was cultured in vitro, and the cell viability, mitochondrial membrane potential, and the level of p-Akt, GSK3β and Caspase-3 protein were measured. For behavior tests, the results showed that Risperidone significantly reversed the Aβ1-42-induced dysfunction in learning, memory, locomotor activity and exploratory behavior. As detected by ELISA assay, risperidone decreased the levels of Aβ1-42, BACE1 and p-Tau in the hippocampus and cortex of AD model mice. Biochemical assay showed that Risperidone reversed the Aβ1-42-induced decrease of cell viability and mitochondrial membrane potential in cultured cortical neurons. The expression of p-Akt was increased, whereas the expression of GSK3β and Caspase-3 were decreased. These results suggested that Risperidone may be used as a promising candidate for AD treatment, for its effects of inhibiting Aβ generation and improving cognitive impairment in mice.

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1. Introduction

Alzheimer's disease (AD), the most common form of dementia among the aged population, is a neurodegenerative disorder characterized by progressive memory and cognitive decline. The pathogenesis of AD is multifactorial, including energetic dyshomeostasis, oxidative stress and neuroinflammation [1–3]. The presence of senile plaques (caused by the deposition of Amyloid β peptide) and neurofibrillary tangles (formed by hyper-phosphorylated tau protein) is an important pathological characteristic which causes neuronal damage [4,5]. Amyloid β peptide (Aβ) may be the most important factor in the formation and development of AD [6–8], and all other pathological characteristics such as protein tau hyperphosphorylation and synaptic loss are linked to Aβ [9]. Aβ is the product of the activity of β-site APP cleaving enzyme1 (BACE1) and γ-secretase upon the amyloid precursor protein (APP), and has been reported to be toxic to hippocampal and cortical neurons [10]. The processing of APP by BACE-1 is the first and rate limiting step in releasing neurotoxic Aβ [11]. Aβ toxicity is mediated by various forms of Aβ. The most toxic form and the major constituent of senile plaques is Aβ1-42, which is often used to established AD model [12]). Besides, tau protein plays an important role in the differentiation and maturation of neurons. The
hyperphosphorylation of tau decreases the ability to copolymerize with tubulin and induces the instability of cytoskeleton [13].

As previously reported, hyper-phosphorylated tau protein is related to the severity of dementia in AD patients [14,15]. However, the mechanism of Aβ-induced toxicity is not completely understood. According to previous reports, Aβ caused the death of neurons through a necrotic or an apoptotic mechanism [16,17]. Aβ-induced damage caused a collapse of mitochondrial membrane potential of neurons. As an important energy-producing organelle, the collapse started the pathway of caspase, which is the intrinsic pathway of apoptosis [18,19]. The activation of caspase-3 is the central event of apoptosis [20]. Glycogen synthase kinase 3β (GSK3β) is a constitutively active kinase, participating in a number of physiological processes ranging from glycogen metabolism to gene transcription. GSK3β also plays a central role in the pathogenesis of AD [21]. According to previous reports, over-activity of GSK3β caused memory impairment and increased Aβ production, tau hyperphosphorylation as well as local plaque-associated microglial-mediated inflammatory responses [22,23]. The serine-threonine kinase, Akt, can prevent the apoptosis by its phosphorylation at Ser473. Activated Akt inactivates GSK3β, thereby inhibiting cell death [24].

Some studies have shown that antipsychotic drugs (APDs), in particular atypical APDs, are associated with the improvement of some cognitive deficits in patients with schizophrenia, especially executive function, spatial memory [25–28]. Antipsychotics are frequently used to treat behavioral and psychological symptoms of dementia (BPSD) among patients with AD [29,30]. Risperidone is the only antipsychotic which is registered for the treatment of BPSD. As previously reported, Risperidone showed better effect on immediate memory, delayed memory and working memory [31,32,25]. However, at present, there is no single study which can explain the potential relationship between Risperidone and Aβ1–42-induced cognitive impairment. Therefore, in this study, we investigated whether Risperidone could inhibit the detrimental effect of Aβ1–42 in the mouse behavioral performance and biochemical parameters or not. In the present study, the behavioral performance was measured by behavioral tests including Morris Water Maze test, Step-through passive avoidance test, Open Field test, Step-Down test, Hole-Board test and Novel object recognition test. Furthermore, the mechanism of Risperidone was explored by various biochemical assessments such as ELISA, MTT, assessment of mitochondrial membrane potential and Western blot analysis.

2. Materials and methods

2.1. Drugs and chemicals

Donepezil hydrochloride was purchased from the Melone Pharmaceutical Co. (Dalian, China). Aβ1–42 was purchased from the Top Science Limited Liability Co. (Houston, USA). Risperidone was purchased from the Topharman Shanghai Co. (Shanghai, China). Coomassie brilliant blue was purchased from BioSharp Co. (Hefei China), Aβ1–42, BACE1 and p-tau Elisa Kits were purchased from R&D Systems Inc.(USA). Mitochondrial Membrane Potential Kit, BCA Assay Kit and MTT Assay Kit were purchased from the Beyotime Biotechnology Co. (Nanjing China). Rabbit anti-β-Caspase3, rabbit anti-p-AKT and rabbit anti-GSK3β were purchased from Signalway Antibody LLC. (Maryland, USA).

2.2. Animals

360 Male ICR mice (18–22 g) were purchased from the Comparative Medicine Centre of Yangzhou University. Before and during the study, animals were housed by group in standard M1 polycarbonate cages (290*178*158 mm) including sawdust as bedding material (five in each cage), provided with food and water free access and kept on a 12-h light/dark cycle (start at 7:00 AM) at constant temperature (22–25 °C) and relative humidity (60 ± 10%).

All experiments were conducted in accordance with the “Guide and Care and Use of Laboratory Animals” (National Institutes of Health) and were approved by the Ethics Committee of China Pharmaceutical University.

2.3. Establishment of AD mouse model

For each behavior test, 60 male ICR mice were randomized into six groups: [A] control group (0.5% Sodium carboxyl methyl cellulose (CMC-Na)), [B] AD model group (Aβ1–42 410 pmol in 5 μl), [C] positive control group (donepezil 1 mg/kg), [D] high-dose Risperidone group (Risperidone-H 4.0 mg/kg), [E] middle-dose Risperidone group (Risperidone-M 2.0 mg/kg), [F] low-dose Risperidone group (Risperidone-L 1.0 mg/kg).

All the six groups were anesthetized with 4% chloral hydrate (400 mg/kg). The animals were set on the stereotaxis instrument for mice. The bilateral hippocampus and cortex were located (AP = −2 mm, ML = 1.5 mm, DV = 1.5 mm from Bregma) [33]. 5 μl normal saline was injected by Hamilton micro-syringe for the control group. For the other five groups, Aβ1–42 (410 pmol in 5 μl) was injected into the bilateral hippocampus. Aβ1–42 was dissolved in sterile normal saline and incubated at 37 °C for a week to obtain the aggregated form before being used. The injection lasted for 2 min, and the needle was left for another 2 min. 48 h after the injection of Aβ1–42, Risperidone was administered intragastrically to the mice of Risperidone-H group (4.0 mg/kg), Risperidone-M group (2.0 mg/kg) and Risperidone-L group (1.0 mg/kg). Donepezil was administered intragastrically to 1 mg/kg. The control group and AD model group were administered with the same dose of CMC-Na. to all groups, reagent was given once daily for 7 days.

2.4. Behavior tests

After 7 days’ administration, six behavioral tests were performed after 7 PM, including Morris Water Maze test, Step-through passive avoidance test, Open Field test, Step-Down test, Hole-Board test and Novel object recognition test. Different mice were used in each behavior test.

2.4.1. Morris water maze test

Spatial learning and memory ability were tested by Morris water maze. Methods for training of animals on the water maze task have previously been described [34–36]. The Morris water maze consisted of a black circular pool (120 cm in diameter and 50 cm in height), filled with water (25 °C, 30 cm in depth). The equipment was placed in a sound proof room with overhead fluorescent lights at 250 lx. The pool was divided into four quadrants. A black platform (9 cm in diameter) was placed in the fourth quadrant and submerged 5 mm below the water surface. The Morris water maze test was consisted of five-days training trial and one-day probe trial. The mice were applied for visible platform training on the first two days of training trial and for hidden platform training on the following three days. For visible platform training, a 5 cm-high flag was set on the platform. Each mouse was brought to each of the four quadrants facing the edge of the pool. If the mouse located the platform and remain on it for 10 s, the test ended automatically. If the mouse did not locate the platform during 90 s, it was placed on the platform for 30 s. For hidden platform training, the flag was removed from the platform. The training method was same with the visible platform training. For the probe trial, the platform was removed. The mice were brought to the
quadrants as the training trial. The mice were placed and swam for 90 s. The time in target quadrant and platform crossings was recorded.

2.4.2. Step-through passive avoidance test
Learning and memory ability were measured by the Step-through passive avoidance test, which consisted of acquisition and retention trials. The test cage consisted of two identically sized compartments, with a guillotine door to separate light and dark. Illumination was available in the light box through LED lights at 250 lx. During the acquisition trial, the mice were placed in the light compartment and were allowed to explore the environment freely for 5 min so that they can get familiar with the environment. Then the door was opened, the mice tried to enter the dark compartment since they preferred to stay in dark place. As soon as the mice came into the dark compartment, an electrical shock was delivered through the steel rods. The training lasted for 5 min. Mice that did not enter the dark compartment within 180 s were excluded from the test. The retention trial was preformed one day after the acquisition trial. The mice were placed into the light compartment and the door was opened [37]. The time that mouse spent to enter the dark compartment was recorded as the latency. The numbers of the mouse entering the dark compartment during 5 min were recorded as error numbers. If the mouse did not cross the door, the latency was identified as 300 s.

2.4.3. Open field test
Before the open field test, a spontaneous experiment with the moving distance of mice within 60 min was measured. The mice were placed in the center of open field equipment, a black box (50 cm × 50 cm × 50 cm) equipped with video-tracking system. The total moving distance of mice in 60 min were automatically recorded.

Locomotion and exploration activity were measured by open field test. Method for the open field test has been previously described [37]. Illumination was available in the light box through fluorescent lights at 300 lx, which was placed at the center of the arena. Each mouse was placed in the center of the box and were allowed to explore the box freely for 5 min. With the ANY-maze software, the total distance, number of line crossings and time of central squares were recorded to evaluate the horizontal locomotor activity.

2.4.4. Step-down test
Learning and memory ability were measured by the Step-through passive avoidance test. The step-down test was carried in the passive avoidance chamber with a steel grid floor. Illumination was available in the light box through LED lights at 250 lx. An insulated platform was placed in bottom right corner of the chamber. For the training trial, the mouse was allowed to get familiar with the chamber for 5 min. Then the power was on and the mouse was placed on the platform. Once the mouse stepped down, it would receive an electric shock, which caused it to return to the platform. The number of the mice stepping down from the platform within 5 min was recorded as errors. 24 h after the training trial, the recall trial was conducted. The mice were placed on the platform with the electric shock. Latency and the error frequency of step-down were recorded.

2.4.5. Hole-board test
Exploration activity were measured by hole-board test. The hole-board test was conducted in a black chamber (25cm × 25cm × 25 cm). The floor of the base had 25 holes of 3 cm equidistant from each other. Illumination was available in the light box through LED lights at 250 lx. The mice were placed on the hole-board and were allowed to explore freely for 5 min. The total number of head dips was recorded.

2.4.6. Novel object recognition test
Memory ability was measured by novel object recognition test. As previously described [38], the test was carried out in an open field chamber (50 cm × 50 cm × 50 cm). Illumination was available in the light box through fluorescent lights at 300 lx, which was placed at the center of the arena. On the previous day to the test, mice were placed in the chamber to get familiar with the environment for 5 min. Object A was a black plastic cube (1 × 5 cm), object B was a white plastic cylinder (r = 3 cm, h = 6 cm). For the first trial, two identical objects (object A) were placed on the diagonal of the open field with a spacing of 35 cm, and the mice were allowed to explore the objects freely for 5 min. Exploration was considered when the head of the mouse was oriented toward the objects with its nose within 2 cm of the object. The second trial was carried out 24 h later. One of the object A was replaced by an object B. The objects and open filed were cleaned only with distilled water thus minimizing the interference of potential unfamiliar smells. The time spent with the two objects was recorded (EA and EB). Results were expressed as discrimination index (DI), DI = (EB − EA)/(EB + EA).

2.5. Tissue preparation for biochemical analyses
After the completion of behavioral tests, mice were killed by decapitation fast. Hippocampus and cortex were removed and homogenized respectively (1:5 w/v) in normal saline and centrifuged at 400g, at 4 ℃ for 30 min. The supernatants obtained were used for the determination of biochemical analyses.

2.6. Protein determination
The protein content was measured by the method of [39] with bovine serum albumin as a standard. Aβ1–42, BACE1 and p-Tau (ser396) levels in the hippocampus and cortex were determined using a commercially available high-sensitive ELISA kit (R&D Systems Inc. USA) following manufacturer instructions. Data are represented as picograms (U) per ml of protein.

2.7. Primary cortical neuron culture
Newborn ICR mice were used to prepare primary cortical cultures as previously described [40]. Primary cortical cells were plated in 6-well plates at a density of 1 × 10⁵ cells per well. Neurons cultured for seven days were used in the experiments. The cells were divided into six groups: [A] control group, [B] AD model group [Aβ1–42 (10 µM),C] positive control group [Aβ1–42 (10 µM)] + Donepezil (10 µM),[D] high-dose Risperidone group[Aβ1–42 (10 µM) + Risperidone (20 µM),E] middle-dose Risperidone group[Aβ1–42 (10 µM) + Risperidone (10 µM),F] low-dose Risperidone group[Aβ1–42 (10 µM) + Risperidone (5 µM)]. Four hours after giving 10 µM Aβ1–42, all the groups were administered with drugs. 24 h later, MITT assay was preformed and neuronal proteins were collected for Western blot analysis.

2.8. Measurement of cell viability
MITT assay was preformed to measure the cell viability. The medium was refreshed by PBS solution for three times. Then 100 µl MITT were added to each well and the plate was incubated for 4 h at 37 ℃. Then the solution was aspirated off, and 100 µl DMSO was added to each well
to solublize the crystals in cells. The optical densities (ODs) were measured at 490 nm against 630 nm with a microplate reader.

2.9. Assessment of mitochondrial membrane potential

Mitochondrial membrane potential was monitored using the Mitochondrion Membrane Potential Kit (JC-1) (Beyotime, Nanjing...
The fluorescence of JC-1 monomer was monitored at an ex/em wavelength of 490/530 nm and JC-1 aggregate at ex/em wavelength of 525/590 nm.

2.10. Western blot analysis

Cell lysate was homogenized in lysis buffer [150 mM NaCl, 50 mM Tris, 10% glycerol, 1% Triton X-100, 100 mM NaF and 10 mM EDTA] containing Protease Inhibitor Cocktail. The lysates were centrifuged at 6500g for 10 min at 4°C and protein was determined by BCA assay kit (Beyotime, Nanjing China). Western blot analysis was performed as previously described [41]. Briefly, samples were denatured in loading buffer and heated for 5 min at 100°C. After the electrophoretic separation, proteins were transferred to nitrocellulose membranes and blocked with 5% skimmed milk at room temperature for 1 h. Then the membranes were incubated overnight at 4°C with the primary antibodies: anti-Caspase38G10(1:1000; Signalway Antibody), anti-p-AKT<sub>Tyr474</sub>(1:1000; Signalway Antibody) and anti-GSK3β<sub>D5C5Z</sub> (1:1000; Signalway Antibody), β-actin (1:2000; Signalway Antibody) was used as an internal control. The membranes were washed with TBST (TBS containing 0.05% tween-20) for 8 min five times and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Washed the membranes again and incubated with detection solution for 2 min. The membranes were scanned using an enhanced chemiluminescence detection system.

2.11. Statistical analyses

Data were expressed as the mean ± SEM. In particular, group differences in the Morris water maze escape latencies were analyzed using a mixed-design analysis of variance (ANOVA) with “days” as the with-in-subject factor and “group” as the between-subject factor. The other data were determined by one-way analysis of variance (ANOVA). Multiple comparison between the groups was performed using S-N-K method. Statistical significance was set at p < 0.05.

Fig. 2. A, B. Effects of Risperidone on the number of errors and latency (s) in the passive avoidance step-through test by the Aβ1-42 induced cognitive impairment. C. The track plots of mice in the Step-through passive avoidance test (one mouse for each group). The data shown are mean ± SEM (n = 10). *P < 0.05, **P < 0.01 vs. model group.
Fig. 3. Effects of Risperidone on the Aβ1-42-induced cognitive impairment and locomotor activity in the open field test. A. Total distance. B. Number of line crossing. C. Time of central squares (s). D. The track plots of mice in the open field test (one mouse for each group). The data shown are mean ± SEM (n = 10). *P < 0.05, **P < 0.01 vs. model group.

Fig. 4. Effects of Risperidone on the (A) number of errors and (B) latency (s) in the step-down test by the Aβ1-42 induced cognitive impairment. The data shown are mean ± SEM (n = 10). *P < 0.05, **P < 0.01 vs. model group.
3. Results

3.1. Effect of Risperidone on Aβ1–42-induced cognitive impairments in morris water maze test

The Morris water maze test was used to measure the effect of Risperidone on the spatial learning and memory ability. During the visible platform training trials, Mice in each group showed similar escape latency (4 trials/mouse/day for 2 days, effect of day, F[5,474] = 34.151, P < 0.001; effect of group, F[5,474] = 2.547, P > 0.05; effect of group-by-day interaction, F[5,474] = 0.995, P > 0.05). Overall statistical comparisons by a repeated measure ANOVA revealed no difference in vision among all the groups. In the spatial hidden- platform variant, and data showed that escape latency pronouncedly increased in model group compared to that of control group (P < 0.05). Treatment with Risperidone, donepezil significantly decreased escape latency (4 trials/mouse/day for 3 days, effect of day, F[5,474] = 19.875, P < 0.01; effect of group, F[5,474] = 5.854, P < 0.01; effect of group-by-day interaction, F[5,474] = 0.842, P > 0.05). During the five-days training trials, the mice in each group showed similar swimming speed (4 trials/mouse/day for 2 days, effect of day, F[5,474] = 2.07, P > 0.05; effect of group, F[5,474] = 1.101, P > 0.05; effect of group-by-day interaction, F[5,474] = 0.875, P > 0.05). The probe test was performed after training test. The percentage of time in target quadrant and time of platform crossing were markedly decreased in model group (P < 0.01), which were revised in donepezil group and Risperidone-H group (F[5,54] = 7.415, P < 0.01; F[5,54] = 7.182, P < 0.01). These results indicated that the ability of spatial learning and memory of mice was seriously impaired by Aβ1–42 and Risperidone could improve this cognitive impairment (Fig. 1).

3.2. Effect of risperidone on Aβ1–42-induced cognitive impairments in step-through passive avoidance test

Injection of Aβ1–42 effectively induced learning and memory deficiency in the Step-through passive avoidance test. Risperidone-H and Risperidone-M groups, however, reversed the Aβ1–42-induced learning and memory deficiency, as evidenced by reduced number of errors and improved latency time (F[5,54] = 8.568, P < 0.01, P < 0.01; F[5,54] = 5.228, P < 0.01, P < 0.05) (Fig. 2).

3.3. Effect of risperidone on Aβ1–42-induced cognitive impairments in open field test

Before the open field test, a spontaneous experiment with the moving distance of mice within 60 min was measured. The distances of control group, Risperidone-H group, Risperidone-M group and Risperidone-L group was 132.4 ± 15.5 m, 123.1 ± 14.9 m, 125.6 ± 18.2 m, 126.3 ± 17.8 m, respectively. Compared to control group mice, there is no significant difference between Risperidone groups and control group. This indicated that effects of Risperidone on Aβ1–42-induced learning and memory impairment in the behavior test are not due to non-specific motor actions.

During the open field test, the locomotion activities of mice were observed. Comparing with the control group, the model group exhibited a significant decrease in total distances, number of line crossings and time of central squares (F[5,54] = 7.974, P < 0.05; F[5,54] = 10.219, P < 0.01; F[5,54] = 8.727, P < 0.01), suggesting that the spontaneous activities of model mice were decreased by Aβ1–42. The Donepezil group, Risperidone-H and Risperidone-M group showed a significant increase in total distances, number of line crossings and time of central squares (P < 0.05). But no significant difference was observed between Risperidone-L and model group (Fig. 3).

3.4. Effect of Risperidone on Aβ1–42-induced cognitive impairments in step-down test

The results of the step-down test showed that a significant difference of both the latency and error times between model group and control group, suggesting that the AD model was successfully established. Compared with the model group, Donepezil group, Risperidone-H and Risperidone-M groups significantly improved the learning and memory performance of Aβ1–42-induced mice (F[5,54] = 7.509, P < 0.05; F[5,54] = 6.312, P < 0.05). However, no significant difference was observed between Risperidone-L group and model group (Fig. 4).

![Fig. 5. Effects of Risperidone on the number of head dips in the hole-board test by the Aβ1–42 induced cognitive impairment. The data shown are mean ± SEM (n = 10). *P < 0.05, **P < 0.01 vs. model group.](image)

![Fig. 6. Effects of Risperidone on the discrimination index in New object recognition task by Aβ1–42 induced cognitive impairment. The data shown are mean ± SEM (n = 10). *P < 0.05, **P < 0.01 vs. model group.](image)
3.5. Effect of Risperidone on Aβ1–42-induced cognitive impairments in hole-board test

Compared with the model group, the control group exhibited a significant increase in the number of head dips (F[5,54] = 10.515, P < 0.01), suggesting that the AD model was successfully established. The Donepezil group and Risperidone-H group showed increased number of head dips compared with model group (P < 0.01). However, the results of Risperidone-M group and Risperidone-L group did not show significant improvement compared with the model group (Fig. 5).

3.6. Effect of Risperidone on Aβ1–42-induced cognitive impairments in novel object recognition test

The Novel object recognition test was performed to evaluate the learning-memory ability. The results showed that Aβ1-42 caused a significant decline (F[5,54] = 9.140, P < 0.01) in Discrimination Index (DI) compared with control group, suggesting that the AD model was successfully established. Moreover, Donepezil group, Risperidone-H group and Risperidone-M group showed a significant increase in DI compared with the model group (p < 0.05; p < 0.01; p < 0.05) (Fig. 6).

3.7. Effects of Risperidone on the expression of Aβ1–42, BACE1 and p-tau in hippocampus and cortex of mice by ELISA

The increase of Aβ1–42 is considered as an important molecular hallmark in AD and is mainly regulated by BACE1. Therefore, the bioactivity of BACE1 could reflect the content of Aβ1–42. As shown in Fig. 7(A, B), the content of Aβ1–42 and BACE1 in the hippocampus and cortex of model group showed a significant increase compared with control group (F[5,54] = 6.516, F[5,54] = 8.887, F[5,54] = 11.780, F[5,54] = 11.662, P < 0.01). Donepezil, Risperidone-H and Risperidone-M dose significantly decreased the Aβ1–42 and BACE1 content in the hippocampus and cortex of mice (P < 0.05). Moreover, Risperidone-L group did not show significant improvement compared with model group. As shown in Fig. 7(C), the expression level of p-Tau in the hippocampus and cortex of model group increased significantly compared with control group.

![Fig. 7](image-url) A–C. Effects of Risperidone on the expression of Aβ1–42, BACE1 and p-Tau in the hippocampus and cortex of mice by ELISA respectively. The data were expressed as Mean ± SEM in triplicates of three experiments. *P < 0.05, **P < 0.01 vs model group.
were remained viability 3.9. 3.8. Effect of Risperidone on Aβ1–42 induced cell death in primary cultured cortical neurons by MTT

The result of MTT showed that Aβ1–42 induced significant cell viability reduction compared with control group (F[5,54] = 15.58, P < 0.01). Both Donepezil and Risperidone-H significantly inhibited the reduction of cell viability induced by Aβ1–42 (P < 0.01). Risperidone-M also increased the cell viability significantly (P < 0.05), while Risperidone-L did not exhibit such effect Fig. 8.

3.9. Assessment of mitochondrial membrane potential by JC-1

JC-1 is a fluorescent probe for the assessment mitochondrial membrane potential. For the photomicrograph of Primary cultured cortical neurons, JC-1 which aggregated in healthy mitochondria generated red fluorescence, and those which remained in cytosol produced green fluorescence. As shown in Fig. 9, green fluorescence in the model group is significantly more than the control group, suggesting that the Aβ1–42 induced adverse effect to mitochondria. Also, Donepezil and Risperidone significantly increased the red fluorescence while the effect was enhanced as the dosage of Risperidone increased.

3.10. Western blot analysis of apoptotic protein

Western blot was performed for the analysis of p-Akt, GSK3β and Caspase3. As the results shown in Fig. 10, compared with control group, Aβ1–42 induced the expression of p-Akt and increased the expression of GSK3β and Caspase3 significantly (F[5,12] = 8.892, P < 0.01; F[5,12] = 6.722, P < 0.01; F[5,12] = 7.812, P < 0.01), which suggested the Aβ1–42-induced change of apoptotic protein. Moreover, Donepezil, Risperidone-H and Risperidone-M

![Fig. 8. Effect of Risperidone on Aβ1–42 induced cell death in primary cultured cortical neurons by MTT. The data were expressed as Mean ± SEM, in triplicates of three experiments. * P < 0.05, ** P < 0.01 vs. model group. n = 3 × 6 repeat holes.](image1)

![Fig. 9. Effect of Risperidone on the loss of mitochondrial membrane potential in primary cultured cortical neurons. Photomicrograph of Primary cultured cortical neurons were tested with JC-1. Mitochondrial membrane potential was measured, JC-1 which aggregated in healthy mitochondria generated red fluorescence, and those which remained in cytosol produced green fluorescence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image2)
Fig. 10. Western blot analysis of apoptotic protein. (A) Western blot of p-Akt, GSK3β, Caspase3 into the active form, β-actin was used as internal control. (B, C, D) Quantitative analysis of p-Akt, GSK3β, Caspase3. Western blot data were representative of three independent experiments with similar result. The data were expressed as Mean ± SEM in triplicates of three experiments, *P < 0.05, **P < 0.01 vs. model group, n = 3.

dosage significantly inhibited the Aβ1–42-induced effect (P < 0.05, P < 0.05).

4. Discussion

In the present study, the pharmacological effect of Risperidone in an Aβ1–42-induced mice model was investigated. We provided some new evidences for the therapeutic effects of Risperidone on cognitive and hippocampal synaptic impairments induced by Aβ1–42 in mice. To the best of our knowledge, this is the first in vivo and in vitro study reporting the beneficial effects of Risperidone in an experimental model of AD based on Aβ1–42-mediated neurotoxicity.

Cognitive impairment and loss of memory are the classical hallmarks of Alzheimer's disease. Six behavioral tests were conducted to evaluate the effect of Risperidone on cognitive function in Aβ1–42-induced AD mice model, including Morris Water Maze test, Step-through passive avoidance test, Open Field test, Step-Down test, Hole-Board test and Novel object recognition test. These behavioral tests are similar in term of hippocampus-dependent tasks. The Morris water maze test is usually used to measure long-term spatial memory [42]. The Step-through passive avoidance test and step-down test are fear-motivated tests to measure the long-term memory in mice models [41]. The open field test is preformed to evaluate the locomotor activity [43]. The hole-board test and Novel object recognition test are commonly used to evaluate the spatial probe and learning-memory ability [44,45]. In Morris water maze test, Risperidone significantly increased the time in both target quadrant and platform crossing time. The increased error time and decreased latency time induced by Aβ1–42 were reversed by Risperidone in the Step-through passive avoidance test and step-down test. In the open field test, when exposed to a new environment, it is typically for mice to show explorative behaviors including increased ambulatory behavior, or
loomation and rearing activity. Both donepezil and Risperidone-H mice showed improved behavioral habitation in the open field tests. The result of spontaneous experiment showed effects of Risperidone on Aβ1–42-induced learning and memory impairment in the behavior test are not due to non-specific motor actions. Cope and Lian reported that Risperidone can reduce the activity levels in mice and rats [46,47]. Our different results might be caused by the mechanism of Risperidone on AD. In our study, there is no evidence altered activity levels confounded any of our results. In the further study, we will try to figure out the effect of Risperidone on the locomotor activity in AD model. In addition, Risperidone increased the exploration activity of mice compared with model group mice in hole-board test. In the novel object recognition test, DI decreased by Aβ1–42 was significantly enhanced by Risperidone. These results clearly indicate that Risperidone reversed Aβ1–42-induced dysfunction in learning, memory, locomotor activity and exploratory behavior.

We designed experiments to explore the possible mechanism of Risperidone therapeutic effect on Aβ1–42-induced cognitive dysfunction. The expression of Aβ1–42, BACE1 and p-Tau in hippocampus and cortex of mice were measured. As the results showed, compared with control group, Aβ1–42 increased the expressions of Aβ1–42, BACE1 and p-Tau in model mice, which are important biochemical indicators of AD. That might be the inner cause of the behavioral abnormality of AD mice. Furthermore, Risperidone significantly decreased the levels of Aβ1–42, BACE1 and p-Tau in the hippocampus and cortex of mice. These results suggest that Risperidone may inhibit the generation of Aβ and improve cognitive impairment by modulating the Aβ pathway.

As the most important factor in the formation and development of AD, the role of Aβ is not yet fully understood. But it is clear that Aβ causes neuron death through two different processes: apoptosis and necrosis [48,49]. Neuronal apoptosis is one of the most obvious consequences of Aβ accumulation, considered as the cause of memory defect [50]. Neuronal lost and degeneration were observed both in the brains of AD patients and the rodent brain injected with Aβ [51]. Our study showed that Risperidone can improve the ability of both long-term and short-term memory and reduced Aβ1–42-induced neuronal damage of cortex in AD model mice. Taken together, we assumed that the beneficial effect of Risperidone on Aβ1–42-induced cognitive impairment was due to the reversion of apoptosis caused by Aβ1–42.

Primary cortical neuron was cultured in vitro and cell viability, mitochondrial membrane potential, and the level of p-Akt, GSK3β and Caspase-3 protein were measured. The data provided further evidence that Risperidone could inhibit neurons apoptosis induced by Aβ1–42. MTT assay showed that Risperidone reversed the Aβ1–42-induced decrease of cell viability in cultured cortical neurons, suggesting that Risperidone has neuroprotective effects. In a previous paper [52], Aβ1–42 induces the apoptotic through multiple effects on mitochondrial function such as energy metabolism failure, caspase-3 activation, a decrease in the amount of p-Akt and an increase in the level of GSK3β. In our culture system, the effect of Aβ1–42 on mitochondrial membrane potential, Caspase-3, GSK3β, mitochondrial membrane potential and p-Akt was the case.

Our present data showed that Risperidone inhibited all such Aβ1–42-induced apoptotic indicators in the primary cortical neuron. Risperidone inhibited the decrease of mitochondrial membrane potential and prevented cell death. Additionally, Risperidone decreased the expression of Caspase-3, an important protein that mediates the mitochondrial apoptotic pathway.

As an antipsychotic, Risperidone is often used in conjunction with anti-Alzheimer drugs to treat the behavioral and psychological symptoms of dementia (BPSD) among AD patients. Devanand et al. reported relapse in AD patients switched from Risperidone to placebo after response to Risperidone treatment [53]. Besides, as reported, continuous use of antipsychotic is frequent in long-term care residents with AD [54]. Some researchers hold the converse opinion on treating patients with Alzheimer’s disease with antipsychotic. As reported by Vigen et al. the antipsychotics were associated with worsening cognitive function of AD patients [55]. The effect of Risperidone on AD patients is still unclear. In our study, Risperidone showed protective effect on Aβ1–42-induced cognitive impairment and neural apoptosis in mice. But it still need to be further studied whether Risperidone has the same beneficial effect in the treatment of patients with Alzheimer’s disease.

5. Conclusions

In conclusion, our study showed that Risperidone could reversed Aβ1–42-induced dysfunction in learning, memory, locomotor activity and exploratory behavior of AD model mice and has neurotherapeutic effect against Aβ1–42-induced damages in the hippocampus and cortex by modulating the Aβ pathway in AD. The results revealed that Risperidone may be used as AD therapeutic drug which requires continuous research efforts for further understanding of the molecular mechanisms operative in AD.

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