Alzheimer’s disease is one of the leading causes of death for those aged 65 years and older (Mebane-Sims and Alzheimer’s, 2009). Alzheimer’s disease is a highly devastating neurodegenerative disorder and is characterized by severe memory and cognitive decline. The deposition of beta-amyloid (Aβ) protein is the primary pathology associated with Alzheimer’s disease. Current treatments for Alzheimer’s disease only offer limited symptomatic alleviation, and more effective therapies are needed for Alzheimer’s disease. Our primary data showed that stemazole, a novel small molecule, protected SH-SY5Y cells from toxicity induced by hydrogen peroxide and Aβ aggregates in vitro. In this study, we evaluated the therapeutic effects of a 14-day stemazole treatment in Aβ1-40 aggregate injection rat model of Alzheimer’s disease. Administration of stemazole reversed learning and memory deficits induced by the Aβ1-40 aggregates in a dose-dependent manner, as assessed by a Morris water maze task. In addition, the number of Aβ1-40 aggregates was reduced in the hippocampus, as demonstrated by micro-positron emission tomography/computerized tomographic scanning. Finally, stemazole treatment reduced degeneration and the loss of neurons in the hippocampus, as shown by histology and immunohistochemical analysis. The neuroprotective effects of stemazole may be important for its therapeutic efficacy. These results demonstrate that stemazole is effective in the treatment of Aβ1-40 aggregates injection rat model, suggesting that this compound may be used as a therapeutic agent against Alzheimer’s disease.

1. Introduction

Alzheimer’s disease is a highly devastating neurodegenerative disorder and is characterized by memory impairment and cognitive decline. Alzheimer’s disease is one of the leading causes of death for those aged 65 years and older (Mebane-Sims and Alzheimer’s, 2009). It is estimated that the number of Alzheimer’s disease patients worldwide will increase from 26.6 million in 2006 to 106.8 million by 2050 (Brookmeyer et al., 2007). Because current treatments for Alzheimer’s disease only offer limited symptomatic alleviation (Jakob-Roetne and Jacobsen, 2009), more effective therapeutic agents are urgently needed. Studies have revealed that the primary pathology associated with Alzheimer’s disease is the formation of abnormal metabolic products of beta-amyloid (Aβ) protein (Selkoe, 1991; Hardy and Higgins, 1992). The Aβ aggregates located around neurons not only have a direct toxic effect on the neurons, but also enhance the susceptibility of neuronal cells to free radicals, nerve toxins and other harmful factors, leading to widespread degeneration and the loss of functional neurons (Cappai and Barnham, 2008; Castellani et al., 2008; Lauren et al., 2009; Lee et al., 2007).

The abnormal metabolism of Aβ initiates a series of events that result in neuronal degeneration and death and ultimately results in memory and cognitive decline. Recently, several compounds, such as curcumin, SK-PC-B70M and T-817MA, which protect neurons from Aβ aggregate-induced toxicity, have been shown to have therapeutic potential for the treatment of Alzheimer’s disease (Hawkes et al., 2009; Sabbagh, 2009; Seo et al., 2009). Some of these compounds have entered clinical trials.

The methods to evaluate the effects of novel compounds on Alzheimer’s disease in animals include behaviour tests, immunohistochemistry, etc. Besides these, we used micro-positron emission tomography/computerized tomographic (micro-PET/CT) imaging in our study. PET/CT as an advanced clinical technique represents real-time non-invasive quantitative method for the diagnosis of Alzheimer’s disease. Among the radiolabeled ligands used to image Aβ aggregates, [11C]2-(4’-(methylamino) phenyl)-6-hydroxybenzothiazole ([11C]PIB) is the most well-known and has been clinically evaluated (Noble and
Scarmeas, 2009). In addition to agents that target Aβ aggregates, 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG), a metabolism imaging agents, is commonly used to detect glucose metabolism in Alzheimer’s disease (Ogawa et al., 2006). The evaluation of candidate compounds for Alzheimer’s disease by micro-PET/CT imaging using [11C]PIB and [18F]FDG in animal models is an effective method, which well coincides with clinical diagnose.

Stemazole (C9H9N5OS2, CA Index Name: Hydrazinecarbothioamide, N-[4-(4,5-dihydro-5-thioxo-1,3,4-oxadiazol-2-yl)phenyl]-) (Fig. 1) is a novel small molecule (Han et al., 2010). Our preliminary study found that stemazole protected SH-SY5Y cells from toxicity induced by H2O2 in vitro (Supplementary materials, Supplementary Fig. 1). We hypothesized that stemazole may act as a neuroprotectant in vivo. To test our hypothesis, we used the Aβ1-40 aggregate injection rat model of Alzheimer’s disease, which has been commonly used in recent studies (Nitta et al., 1997; Tang et al., 2008; Zheng et al., 2008; Wang et al., 2001), and evaluated the efficacy of stemazole. Techniques used in the study included the Morris water maze task, micro-PET/CT, and tissue histology and immunohistochemistry.

2. Materials and methods

2.1. Chemicals

Stemazole was synthesized by the Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University. The purity of stemazole was determined to be >98% by high performance liquid chromatography (HPLC), and the structure of stemazole was confirmed by mass spectrometry and nuclear magnetic resonance. Human Aβ1-40 protein was purchased from Bachem AG (Bubendorf, Switzerland). Galantamine hydrobromide injection (2.5 mg/ml, lot number: H31020672) was purchased from Suzhou Sixth Pharmaceutical (Jiangsu, China).

2.2. Animals

Male Wistar-Unilever rats (Vitalriver, Beijing, China), weighing 180–200 g at the beginning of the experiment, were housed in a room maintained at 23 °C with a 12-hour light-dark cycle. The rats were allowed free access to food and water except during the memory test. All experimental procedures were performed in accordance with National Institutes of Health Guidelines for the care and use of laboratory animals.

2.3. Surgery and drug administration

Rats were randomly divided into two groups for stereotaxic surgery. A sham-operated group (n = 8) received an injection of an isotonic saline solution. A second group (all remaining animals) was injected with Aβ1-40 aggregates into the hippocampus. Before injection, the Aβ1-40 peptide was dissolved in a 0.9% saline solution at a concentration of 5 mg/ml and incubated at 37 °C for one week to induce aggregation (Zheng et al., 2008).

Animals were anesthetized with 0.35 g/kg chloral hydrate intraperitoneally and mounted in a stereotaxic apparatus (Stoelting, USA). Aβ1-40 aggregates (2 μl) or vehicle was administered slowly over 5 min into the unilateral (right) hippocampus by cerebral ventricle injection at the following coordinates: −3.0 mm anteroposterior, −2.0 mm later to bregma, and −3.3 mm ventral to the skull surface.

Two weeks were allowed for recovery from surgery. After the first Morris water maze test (15–19th day after surgery), Aβ1-40 aggregate-injected animals were randomly assigned to five groups (vehicle, galantamine, stemazole (3 mg/kg), stemazole (10 mg/kg) and stemazole (30 mg/kg); n = 8 for each group). Rats in each group were injected with 0.9% saline, galantamine (2.5 mg/kg), stemazole (3 mg/kg), stemazole (10 mg/kg), and stemazole (30 mg/kg), respectively. All injections were intraperitoneal and were given once per day for 14 consecutive days.

2.4. Morris water maze task

Spatial learning memory was assessed by the Morris water maze behavioural test. The water maze apparatus (Chinese Academy of Medical Sciences, China) consisted of a circular pool (150 cm diameter, 60 cm high) divided into four equally spaced quadrants. A translucent platform, submerged 1.5 cm below the surface of the water, was hidden in the centre of quadrant II during the training period and was then removed at the time of the probe task.

Memory training was conducted twice a day for four consecutive days before every probe task. Each rat was allowed to swim until it found the platform or until 120 s elapsed. Then the rat was left on the platform for 10 s. The probe tasks were performed on the 19th day following the operation and the 5th day following the last drug administration. During the probe task, rats were allowed to swim for 60 s in the pool without the platform. The time spent in the probe quadrant was recorded by a computer-based image analyzer.

2.5. Micro-PET/CT imaging

[11C]PIB and [18F]FDG were prepared by the Department of Nuclear Medicine, General Hospital of the People’s Liberation Army (Beijing, China). [11C]PIB was synthesized according to the literature (Zhang et al., 2008), and the radiochemical purity exceeded 97%. [18F]FDG was synthesized according to a standard procedure, and the radiochemical purity exceeded 95%.

Micro-PET imaging was performed using a micro-PET xploRE VISTA-CT scanner (GE, Spanish), which was equipped with Vista Applications software for data acquisition, sinogram generation and image reconstruction. The scanner has a computer-controlled bed and field of view (FOV) (6.8 cm transaxial and 4.8 cm axial) with an image resolution of <1 mm.

[11C]PIB and [18F]FDG micro-PET/CT imaging was performed on three animal groups (with three randomly selected rats in each group): vehicle, stemazole (10 mg/kg) and stemazole (30 mg/kg). Rats were anesthetized with 0.35 g/kg chloral hydrate 30 min after injection of 148.2 ± 11.7 MBq [11C]PIB or 40 min after injection of 90.3 ± 8.1 MBq [18F]FDG via the tail vein. A 15 min static micro-PET/CT scan (10 min static PET scan, and 5 min CT scan) was then performed. Images were reconstructed using a maximum posteriori probability algorithm. Corrections for dead time, random scattering, and attenuation were made for each scan.

The drawing region of interest (ROI) analytical technique was used to obtain the averaged pixel counts of each PET image. Briefly, three adjacent hippocampal slices were selected from every brain, and the area of the hippocampus was determined manually according to the CT scans and magnetic resonance imaging (Inui-Yamamoto et al., 2010 and Meng et al., 2003). The radioactive counts per pixel were automatically determined. The activity difference value (ADV) between the right and left hippocampus was calculated as follows: ADV = AR/AL, where AR represents the uptake value of radioactivity in the left hippocampus and AL represents the uptake value of radioactivity in the right hippocampus. This value was used to evaluate the
extent of radiotracer uptake, which served as a proxy for the amount of Aβ1-40 aggregates in the hippocampus.

2.6. Histology and immunohistochemistry

Following 14 days of drug treatment and behaviour evaluation, three random rats from each group were deeply anaesthetized and sacrificed by perfusion with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (pH = 7.4). The brains were removed, fixed and embedded with paraffin. The paraffin-embedded brains were cut into serial coronal (5 μm) sections, each representing different antero-posterior levels of the hippocampus.

Sections were deparaffinized, rehydrated and stained with hematoxylin-eosin (HE). HE staining showed the morphology of cells within the dentate gyrus of the hippocampus near the injection site. Neurons in the Sector 1 of the cornu ammonis (CA1) region which contains many functional neurons were detected by immunohistochemistry using a monoclonal anti-βIII tubulin (1:100 dilution, Chemicon, USA). Sections were subjected to antigen retrieval in citrate buffer (pH = 6.0), soaked in 3% H2O2 to block the endogenous peroxidase activity, and incubated with an anti-βIII tubulin monoclonal antibody overnight at 4 °C. A peroxidase/DAB kit (EnVision™ Detection System, Dako, USA) was used with diaminobenzidine as the chromogen to visualize immunoreactivity.

Sections were observed under the light microscope with a constant bulb temperature. Immunostained hippocampal cells were counted by an observer blinded to the experimental information. Three sections per brain of the same magnification (400×) were used for quantitative analysis. Damaged granular cells stained by HE in the dentate gyrus and βIII tubulin-positive cells in CA1 were counted. The total cell count for each animal was used to calculate mean cell number of the group and standard errors. The mean cell number of the vehicle group was defined as 100% and used as the baseline; results were calculated as a percentage of this baseline. The images were captured using a digital DP70 colour camera (Olympus, Japan) connected to a BX-50 microscope (Olympus, Japan).

Fig. 2. Stemazole improves memory deficits induced by an injection of aggregated Aβ1-40. The percentage of time spent in the probe quadrant was significantly higher after galantamine, stemazole (10 mg/kg) and stemazole (30 mg/kg) treatment in comparison to vehicle-treated rats. Values represent the mean ± S.E.M (n = 8). **p < 0.001 vs. sham-operated group (independent-sample t-test); *p < 0.05, ***p < 0.001 vs. vehicle group (one-way ANOVA analysis with Dennett’s post hoc test).

Fig. 3. [11C]PIB micro-PET/CT rat brain imaging. Micro-PET/CT images were acquired 30 min after injection of 148.2 ± 11.7 MBq [11C]PIB via the tail vein. Right, middle and left images represent the CT, PET and PET/CT fusion images of the axial rat brain, respectively. In these micro-PET images, increased radioactive uptake was found in the right hippocampus of vehicle-treated group, whereas 30 mg/kg stemazole administration significantly diminished the difference in uptake between the left and right hippocampus.

2.7. Statistical analysis

Data are presented as the mean±S.E.M. Differences between groups were analyzed using a one-way ANOVA followed by the Dunnett’s post-hoc test. The Student’s t-test was used to compare two sets of data. P<0.05 was considered a statistically significant level. All tests were performed using SPSS software (version 13.0).

3. Results

3.1. Stemazole improved memory impairment induced by the infusion of Aβ1-40 aggregates into the rat hippocampus in a dose-dependent manner

Learning and retention of a Morris water maze task was used to evaluate rat spatial memory. As shown in Fig. 2, Aβ1-40 aggregate-injected rats displayed a significant decrease in the swimming time percentage in the probe quadrant compared to the sham-operated group (P<0.001). The rats treated with galantamine alone or with stemazole at the dose of 10 and 30 mg/kg (n=8) swam longer in the target quadrant (26.7±2.0% (P<0.001), 23.3±1.9% (P<0.05) and 27.6±1.9% (P<0.001), respectively) than the vehicle-treated rats (n=8) (17.0±1.5%). These results suggest that stemazole dose-dependently improved spatial memory impairment induced by an infusion of aggregated Aβ1-40 into the right hippocampus of the rat.

3.2. Stemazole reduced Aβ1-40 aggregates in a rat model of Alzheimer’s disease

An animal self-control method (Aβ1-40 aggregates were injected into the right hippocampus, the left hippocampus served as a control) was used for micro-PET/CT imaging. Aβ1-40 aggregation and glucose metabolism were detected using [11C]PIB and [18F]FDG, respectively. [11C]PIB distribution within the hippocampus is shown in Fig. 3. Images of vehicle-treated animals indicated an unsymmetrical distribution of the radiotracer in the left and right hippocampus with an increased uptake of radiotracer in the right hippocampus (the side of injection). Stemazole administration at a dose of 10 mg/kg did not alter the asymmetric distribution of the [11C]PIB radioactive tracer; however, administration of a 30 mg/kg dose significantly decreased the difference in radioactive uptake between the right and left hippocampus. The ADV (AR/AL) of the stemazole 10 mg/kg and 30 mg/kg treatment groups was 1.27±0.08 and 1.01±0.04 (P<0.05), respectively, compared with the ADV of the vehicle-treated group (1.23±0.03) (Fig. 5A). The results from [11C]PIB micro-PET imaging indicated that stemazole administration decreased Aβ1-40 aggregates in the hippocampus.

[18F]FDG hippocampal distribution is shown in Figs. 4 and 5B. We found no significant difference between the right and left hippocampus in vehicle-treated rats, suggesting no decrease in the level of glucose metabolism in the right hippocampus of the rat model. Therefore, [18F]FDG micro-PET imaging was not suitable to evaluate the efficacy of stemazole in the Aβ1-40 aggregate injection rat model.

Fig. 4. [18F]FDG micro-PET/CT rat brain imaging. Micro-PET/CT images were acquired 40 min after injection of 90.3±8.1 MBq of [18F]FDG via the tail vein. Right, middle and left images represent the CT, PET and PET/CT fusion images of the axial rat brain, respectively. In these micro-PET images, no significant difference in uptake of [18F]FDG was found between the right and left hippocampus in both the vehicle-treated group and the two stemazole-treated groups.

3.3. Stemazole reduced neuronal damage in the dentate gyrus and CA1

HE staining and immunohistochemistry using a βIII tubulin monoclonal antibody were used to examine the neuroprotective effect of stemazole.

HE staining data are shown in Figs. 6 and 8A. Compared with the sham-operated group, the Aβ1-40 aggregate-injected rats displayed pathological features of nucleoli ambiguity, circumscription (between nuclei and cytoplasm), obfuscation and cytoplasm diffusion (Fig. 6). The mean number of neurons in the vehicle-treated rats was defined as 100% and used as the baseline. As shown in Fig. 8A, there were a significantly higher number of damaged neurons in the vehicle group as compared to the sham-operated group (100.0±14.3% vs. 5.8±2.6%) (P<0.001). The group treated with stemazole (10 mg/kg and 30 mg/kg) showed a reduced number of damaged cells (22.9±6.8% and 52.2±2.2%, respectively) (both P<0.05). The number of damaged neuronal cells in the galantamine group decreased to 41.8±25.5%, which was not significantly different from the vehicle group. In conclusion, we found that stemazole treatment reduced the number of damaged neuronal cells and ameliorated the pathological changes induced by Aβ1-40 aggregates.

The number of βIII tubulin-positive cells in the CA1 region of the hippocampus was determined. We found an extensive loss of CA1 neurons in the vehicle-treated group. Neither stemazole nor galantamine treatment showed evident loss of neurons (Fig. 7). The mean number of neurons in the vehicle-treated rats was defined as 100%. As shown in Fig. 8B, there was a greater amount of neuron loss in the vehicle-treated group (100.0±11.4%) (P<0.001) compared to the sham-operated group (148.8±5.9%). Stemazole (30 mg/kg) and galantamine treatment markedly prevented neuron loss (152.2±11.2% (P<0.001) and 139.7±21.2% (P<0.05), respectively). Stemazole (30 mg/kg) treatment attenuated the loss of neurons in the CA1 of the Aβ1-40 aggregate-injured rats.

4. Discussion

This is the first report on the neuroprotective bioactivity of stemazole in vitro and in vivo. We evaluated the therapeutic efficacy of stemazole in Aβ1-40 aggregate-injured rats. After a 14-day treatment of stemazole spatial memory impairment was improved in both the 10 mg/kg and 30 mg/kg stemazole-treated groups, as assessed by the Morris water maze task. In addition, the number of Aβ1-40 aggregates in the hippocampus was significantly reduced in the 30 mg/kg stemazole group, as shown by [11C]PIB PET/CT imaging. Furthermore, the number of damaged neuronal cells in the dentate gyrus was reduced in both the 10 mg/kg and 30 mg/kg stemazole-treated groups, as shown by histological analysis; the loss of neurons in the CA1 significantly decreased in the 30 mg/kg stemazole group, as indicated by immunohistochemistry. The neuroprotective effects of stemazole against cytotoxicity induced by Aβ1 aggregates were confirmed in vitro (Supplementary Fig. 2A and B). The methodology
areas in A–E (Bar=200 μm). F and G, galantamine group; (D, I) stemazole (10 mg/kg) group; (E, J) stemazole (30 mg/kg) group. F–J (Bar = 50 μm) are higher magnification images of the boxed areas in A–E (Bar = 200 μm).

and data for the assessment of stemazole neuroprotection of SH-SY5Y cells from toxicity induced by H2O2 and Aβ aggregates in vitro are shown in supplementary materials and supplementary figures.

It has been proposed that Aβ1-40 aggregates play an important role in the pathogenesis of Alzheimer’s disease (Cappai and Barnham, 2008; Lauren et al., 2009). Numerous publications have used the Aβ1-40 aggregate injection rat model to study Alzheimer’s disease (Nitta et al., 1997; Yang et al., 2008; Wang et al., 2001; Yamada and Nabeshima, 2000). These reports demonstrated that an acute infusion of Aβ1-40 aggregates into the brain causes brain dysfunction, as evidenced by neurodegeneration, and an impairment of learning and memory. In our study, we used the Aβ1-40 aggregate injection model to induce memory deficits and test the therapeutic efficacy of stemazole. Aβ1-40 aggregates induced the degeneration of granular cells in the dentate gyrus and a loss of neurons in the CA1. The spatial memory of Aβ1-40 aggregate injected rats declined compared to the sham-operated rats. These results were in agreement with previous studies testing the effects of hippocampal Aβ aggregate injections (Nitta et al., 1997; Tang et al., 2008). Another advantage of using a unilateral Aβ1-40 aggregate injection model is that it uses self-control animal to minimize inter-animal differences. In our model, Aβ1-40 aggregates were injected into the right hippocampus of rats and the left hippocampus served as a control. This self-control method increased the accuracy and objectivity of our quantitative analysis using micro-PET/CT imaging.

Galantamine, an acetylcholinesterase inhibitor, is currently used to treat Alzheimer’s disease patients. However, additional effects of galantamine have been discovered. Arias et al. (2004) observed that galantamine prevented cell death induced by the peptide Aβ1-42 in a concentration-dependent manner. Furthermore, treatment with galantamine significantly improved cognitive dysfunction in the Aβ1-40 mouse injury model (Takeda et al., 2009). Therefore, galantamine was chosen as a positive control to evaluate the therapeutic efficacy of stemazole.

The galantamine treatment showed a significantly lower percentage of damaged cells in the dentate gyrus compared to the vehicle-treated group. (B) An extensive loss of neurons in the CA1 region was observed in vehicle-treated group. Stemazole (30 mg/kg) treatment and positive drug treatment significantly attenuated neuronal loss. The average number of neurons in the vehicle-treated group was defined as 100% and used as the baseline. Values represent the mean ± S.E.M (n = 3). ##P < 0.001 vs. vehicle group (one-way ANOVA analysis with Dennett’s post hoc test).

**Fig. 7.** Representative images of βIII tubulin immunostaining. Significant neuronal loss was observed in the vehicle-treated rats. Stemazole treatment attenuated the loss of neurons in the CA1 in the Aβ1-40 aggregate-injured rats. A similar efficacy was observed in the galantamine-treated group: (A, F) sham-operated group; (B, G) vehicle group; (C, H) galantamine group; (D, I) stemazole (10 mg/kg) group; (E, J) stemazole (30 mg/kg) group. F–J (Bar = 50 μm) are higher magnification images of the boxed areas in A–E (Bar = 200 μm).

**Fig. 8.** Quantitative analysis of HE staining (A) and βIII tubulin immunohistochemistry (B). (A) Significant degeneration of neuronal cells in the dentate gyrus was observed in the vehicle-treated group due to toxicity induced by the Aβ1-40 aggregates. Stemazole treatment showed a significantly lower percentage of damaged cells in the dentate gyrus compared to the vehicle-treated group. (B) An extensive loss of neurons in the CA1 region was observed in vehicle-treated group. Stemazole (30 mg/kg) treatment and positive drug treatment significantly attenuated neuronal loss. The average number of neurons in the vehicle-treated group was defined as 100% and used as the baseline. Values represent the mean ± S.E.M (n = 3). ##P < 0.001 vs. sham-operated group (independent-sample t-test); *P < 0.05, **P < 0.001 vs. vehicle group (one-way ANOVA analysis with Dennett’s post hoc test).
amount of Aβ1-40 aggregates in the hippocampus of the rats model. In addition, it also suggested that quantitative micro-PET imaging not only could be used to measure Aβ1-40 deposits but also to evaluate the therapeutic effects of novel compounds. We determined glucose metabolism in the hippocampus of rats using the [18F]FDG radiotracer in this study (Fig. 4). However, a significant difference between the right and left hippocampus was not found in the vehicle-treated rats. Although many publications have confirmed that [18F]FDG is useful to diagnose early Alzheimer's disease, Kuntner et al. (2009) found that [18F]FDG could not detect regionally decreased FDG binding in Tg2576 transgenic mice. Notably, there are many other factors affecting glucose metabolism. In our study, the level of glucose metabolism in the right hippocampus of the Aβ aggregate-injected rat did not decrease. We speculate that an acute injection of Aβ1-40 aggregates into healthy adult rats may cause a compensation of glucose metabolism after the lesion, which is different from human idiopathic Alzheimer’s disease, a progressive and degenerative disease. This decrease in glucose metabolism was not detected in vehicle-treated rats, and we were unable to evaluate the efficiency of stemazole using [18F]FDG micro-PET imaging.

In this study, histology and immunohistochemistry techniques, which are commonly used methods of clinical diagnosis, were performed to examine the neuroprotective effects of stemazole. HE staining was used to study cell morphology and βIII tubulin was used to label functional neurons in the hippocampus. We observed degeneration of granular cells in the dentate gyrus and loss of neurons in the CA1 of the vehicle-treated rats. Stemazole treatment significantly reduced the number of damaged granular cells and neuronal loss induced by Aβ1-40 aggregate injection (Fig. 8A, B), which suggested that stemazole had a neuroprotective effect in the rat model. Moreover, pathological examination of various primary organs (heart, liver, spleen, lung, kidney, stomach, intestine, thymus and lymph node) of the rats after injection of 30 mg/kg stemazole confirmed the lack of toxicity (analysis was given by Pathology Center of Peking University, China) (Supplementary Fig. 3).

In conclusion, stemazole not only protected SH-SY5Y cells from H2O2 and Aβ42-aggregate-induced cytotoxicity in vitro (Supplementary Figs. 1 and 2), but also protected neurons from toxicity induced by Aβ1-40 aggregates in vivo. The neuroprotective effects of stemazole may be important for its therapeutic efficacy. As a result, stemazole neuroprotection prevented neuron degeneration and reduced neuron loss, the number of Aβ1-40 aggregates decreased and the spatial memory of rats improved. Stemazole exhibited a primary therapeutic effect in an Aβ1-40 aggregate injection animal model of Alzheimer’s disease. These results suggest stemazole may be used as a novel therapeutic agent against Alzheimer’s disease; however, additional mechanistic studies are needed.

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