Neurosci Bull October 1, 2012, 28(5): 532–540. http://www.neurosci.cn
DOI: 10.1007/s12264-012-1258-y

·Original Article·

Schizandrin prevents dexamethasone-induced cognitive deficits

Xiao Xu[1,2], Xing Zhou[1], Xiao-Wen Zhou[1], Zheng Zhang[1], Min-Jing Liao[1], Qi Gao[3], Huan-Min Luo[1,3]

[1]*Department of Pharmacology, School of Medicine, Jinan University, Guangzhou 510632, China*
[2]*Department of Physiology, School of Medicine, Henan University, Kaifeng 475000, China*
[3]*The Joint Laboratory of Brain Function and Health, Jinan University and the University of Hong Kong, Jinan University, Guangzhou 510632, China*

Abstract: Objective To model glucocorticoid-induced cognitive impairment and evaluate the neuroprotection by schizandrin (Sch) against dexamethasone (Dex)-induced neurotoxicity *in vivo* and *in vitro*. **Methods** Cerebral cortical cells from neonatal Sprague-Dawley rats (within 24 hours after birth) were cultured for 9 days, and then treated with Dex (10^{-4}, 10^{-5}, 10^{-6} or 10^{-7} mol/L) for 24 h or pretreated with 10^{-4} mol/L Dex for 24 h followed by 10, 20, 40, or 80 μmol/L Sch for 48 h. Cell viability was assessed using the MTT assay. Immunofluorescence and real-time PCR for MAP2 were performed to confirm the effects of Dex on neurite outgrowth. *In vivo*, kunming mice were randomly divided into six groups: control [(intragastric (i.g.) vehicle for 42 days]; Dex group I (5 mg/kg·d^{-1} Dex i.g. treatment for 28 days followed by i.g. vehicle for 14 days); Dex group II (Dex i.g. for 42 days); Dex + Sch (Dex i.g. for 28 days followed by 5, 15, or 45 mg/kg·d^{-1} Sch i.g. for 14 days). Learning and memory were assessed by Morris water maze test. Histological examination was used to assess pathology and apoptosis in neurons. **Results** Compared to the Dex groups, Sch increased cell viability in a dose-dependent manner, improved performance in the Morris water maze and ameliorated the morphological changes. **Conclusion** Sch has neuroprotective effects against insults induced by glucocorticoid.

Keywords: neuron; glucocorticoid; cognitive impairment

1 Introduction

Glucocorticoids (GCs) are released from the adrenal cortex and regulated by the hypothalamic-pituitary-adrenal axis. They are a class of steroid hormones and play an important role in the stress responses. They can readily cross the blood-brain barrier and participate in many of the central nervous system (CNS) functions by binding to mineralocorticoid and glucocorticoid receptors. While physiological levels of glucocorticoid facilitate long-term potentiation[1,2] and enhance learning and memory[3], too low or too high levels may cause impairment. In addition, stress or glucocorticoid levels may affect hippocampus-dependent learning and synaptic plasticity, following the 'inverted U-shaped' curve[4-7].

Dexamethasone (Dex) is a synthetic glucocorticoid-like compound, and has been used to induce neuronal impairment in primary cultured cortical neurons and *in vivo*. Schizandrin (Sch) is one of the active dibenzocyclooctadiene lignans, isolated from the fruit of *Schisandra chinensis* (Turcz.) Baill, a tonic in traditional Chinese medicine. Sch has been reported to possess various biological properties,

Corresponding author: Huan-Min Luo
Tel & Fax: +86-20-85220160
E-mail: tlhm@jnu.edu.cn
Article ID: 1673-7067(2012)05-0532-09
Received date: 2012-01-09; Accepted date: 2012-06-04

including anti-diabetic and anti-oxidative stress effects, promoting the immune response, and hepatoprotection, as well as a neuroprotective effect against glutamate-induced neuronal excitotoxicity[8-12]. To date, there is no report on the neuroprotective effect of Sch against Dex-induced CNS deficits. Therefore, the present study was designed to evaluate the neuroprotective effects of Sch against Dex exposure both *in vitro* and *in vivo*.

2 Materials and methods

2.1 Materials and reagents Sch was obtained from the Guangdong Institute for Drug Control (Guangzhou, China). Dulbecco's modified Eagle's medium nutrient mixture F-12 (DMEM/F12), fetal bovine serum (FBS) and B27 supplement were from Gibco (Carisbad, CA). Dex, dimethyl sulfoxide (DMSO), poly-*L*-lysine, anti-MAP2 antibody and 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) were from Sigma (St. Louis, MO). Cy3 AffiniPure goat anti-rabbit IgG was from Proteintech Group Inc. (Chicago, IL). Prime script™ RT reagent kit and SYBR® Premix Ex Taq™ kit were from Takara (Tokyo, Japan). Cell culture plates were from Jet Biofil (Canada).

2.2 Cell culture and treatment Cerebral cortical cells were obtained from neonatal Sprague-Dawley rats (within 24 h after birth). Rats were decapitated and brains were immediately removed. The cerebral cortex was dissected and placed into cold D-Hank's solution. After removal of meninges, the tissue was minced and incubated at 37ºC for 10 min in D-Hank's solution containing 0.25% trypsin. Digestion was terminated with DMEM/F12 supplemented with 10% FBS. The cell suspensions were centrifuged (1 000 r/min, 10 min), resuspended in DMEM/F12 supplemented with 0.4% B27, and plated onto 96-well poly-*D*-Lysine-coated dishes at a density of 3×10^4/mL. After 6 h, the medium was replaced by DMEM/F12 supplemented with 2% (*v/v*) B27. Cultures were kept at 37ºC in a 5% CO_2 atmosphere. Half the medium was changed every 3 days with fresh medium. Cortical neurons were cultured for 9 days, and then underwent treatment with Dex and/or Sch. All procedures were approved by the Laboratory Animal Administration Committee of Jinan University.

To determine its neurotoxicity, Dex was dissolved in DMSO at 0.1, 10^{-2}, 10^{-3} and 10^{-4} mol/L and further diluted with cell culture medium (DMEM/F12 supplemented with 2% B27). The final concentration of DMSO in cell culture medium was 0.1%. On the day of experiment, medium was replaced with fresh culture medium containing Dex at a final concentration of 10^{-4}, 10^{-5}, 10^{-6} or 10^{-7} mol/L, and then incubated for 24 h, followed by assessment of neurotoxicity.

Sch was dissolved in DMSO at 10, 20, 40 and 80 mmol/L and further diluted with cell culture medium (DMEM/F12 supplemented with 2% B27). Neurons were pre-treated with 10^{-4} mol/L Dex for 24 h, and then with different concentrations of Sch for 48 h. Then the neurons were assessed for viability.

2.3 Animals and grouping Ninety Kunming (KM) mice (male, 4 months old, 35 ± 5 g) from the Center of Laboratory Animals of Guangdong were housed at 22–25ºC under a 12:12 h light/dark cycle, with access to food and water *ad libitum*. Mice were weighed every 2 days. All procedures were approved by the Laboratory Animal Administration Committee of Jinan University and performed according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No.85–23, 1985, revised 1996).

The mice were randomly divided into six groups (n = 15/group): control [intragastric (i.g.) vehicle i.g. for 42 days]; Dex group I (5 mg/kg·d^{-1} Dex i.g. for 28 days followed by i.g. vehicle for 14 days); Dex group II (Dex i.g. for 42 days); and three Dex + Sch groups (Dex i.g. for 28 days followed by 5, 15, or 45 mg/kg·d^{-1} Sch i.g. for 14 days). Dex and Sch were suspended in distilled water for administration. To avoid effects on the behavioral tests, the solutions were administered at 17:00 and the tests were performed the next day between 08:00 and 16:00.

2.4 Cell survival assay Cell viability was assessed using the MTT assay. After neurons were treated for 72 h, the medium was replaced by DMEM/F12 with MTT (10:1, *v/v*; MTT was first prepared at 0.5 mg/mL in PBS). After incubation for 4 h, the medium was removed and DMSO (150 μL) was added to each well to dissolve the formazan. Absorbance was measured at 570 nm using a Bio-Rad 400 microplate reader (Bio-Rad, Hercules, CA).

2.5 Microtubule-associated protein 2 (MAP2) immunofluorescence Cells were fixed with 4% paraformaldehyde for 20 min at room temperature, then permeabilized with 0.1% TritonX-100/PBS for 10 min. Then, cells were blocked with 1% bovine serum albumin for 30 min and incubated overnight at 4ºC with anti-MAP2 antibody (1:800, diluted in blocking solution). After three washes in 0.1 mol/L PBS, cells were incubated with Cy3 AffiniPure goat anti-rabbit IgG (1:80, diluted in 0.1 mol/L PBS). Images were acquired on a fluorescence microscope (Olympus model IX-71; 400× magnification; Tokyo, Japan).

2.6 Real-time PCR analysis of MAP2 mRNA After neurons were treated with Dex for 24 h, total RNA was extracted with RNAiso Plus (Takara), and cDNA was generated by PrimeScript™ RT reagent kit (Takara). Then real-time PCR was performed by a LightCycler 480 Real-Time PCR System (Roche, Basal, Switzerland): 95ºC (predenaturation) for 30 s, and 45 cycles of denaturation at 95ºC for 10 s, annealing at 58ºC for 10 s, and extension at 72ºC for 10 s. GAPDH was used as the internal control. All samples were tested in triplicate.

2.7 Morris water maze test The maze (50 cm in height and 110 cm in diameter) was divided into four quadrants by four fixed points, and filled with water to a depth of 30 cm. The water temperature was maintained at 22–25ºC. An 8-cm diameter escape platform was kept in one quadrant of the pool and submerged 1 cm below the water surface. The platform had the same color as the tank and the water was rendered opaque with nontoxic ink. Mice were trained to find the hidden platform according to the spatial cues in the pool. Each mouse was placed gently at a start point in the middle of the rim of a quadrant, facing the wall, and was allowed 60 s to find the platform. After finding the platform, the mouse was allowed to stay on the platform for 15 s. If the mouse failed to find the platform, it was manually guided to it and allowed to stay there for 15 s. Each mouse had two trials per day for 5 successive days (from day 23 to day 27, or from day 37 to day 41 of treatment). On day 6, 24 h after the previous trial, the platform was removed from the maze and mouse was allowed to swim for 60 s. During the experiment, each mouse was monitored by a digital camera 80 cm above the maze and parameters were analyzed using the DigBehav 3.0 system (Jiliang Software Technology Co. Ltd., Shanghai, China).

2.8 Histological analysis Mice were anesthetized with 0.3% (*w/v*) pelltobarbitalum natricum (0.1 mL/10 g body weight) and perfused through the heart with 0.1 mol/L PBS, followed by 4% paraformaldehyde in 0.1mol/L PBS. The brain was immediately removed and transferred to 4% paraformaldehyde at 4ºC. After cutting along the midline, each brain was embedded in paraffin and cut into 3-μm sections on a microtome. The sections were stained with hematoxylin and eosin (HE) and examined by a microscope (Olympus model IX-71). Cells that displayed small soma and were deeply stained were counted.

2.9 Data analysis All data are expressed as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni or LSD *post hoc* test. P <0.05 was considered statistically significant.

3 Results

3.1 Neurotoxicity of Dex MTT assays showed that 10^{-5} and 10^{-4} mol/L Dex decreased neuron viability (84.73 ± 2.37% and 72.22 ± 1.98% *vs* 100.00 ± 1.68% in control, both P <0.01), while lower doses (10^{-6} and 10^{-7} mol/L) had no effect (95.74 ± 2.09% and 96.62 ± 2.53%). In addition, MAP2 immunofluorescence staining showed that the neurites of neurons treated with 10^{-4} mol/L Dex were decreased in number and shorter than those of control (Fig. 1). Real-time PCR of MAP2 mRNA (Fig. 2) further demonstrated that 10^{-6}, 10^{-5} and 10^{-4} mol/L Dex decreased MAP2 mRNA expression compared with control (P <0.01).

3.2 Sch attenuated Dex-induced toxicity in primary cultures of rat cortical cells After treatment with Dex (10^{-4} mol/L) for 24 h, 10, 20, 40, or 80 μmol/L Sch was added to the culture medium for 48 h. The results showed that 20, 40, and 80 μmol/L Sch were neuroprotective against Dex-induced toxicity in a dose-dependent manner (Fig. 3, Table S1).

3.3 Sch alleviated the cognitive impairment induced by Dex The results of the Morris water maze showed that Dex treatment for 28 days impaired learning and memory

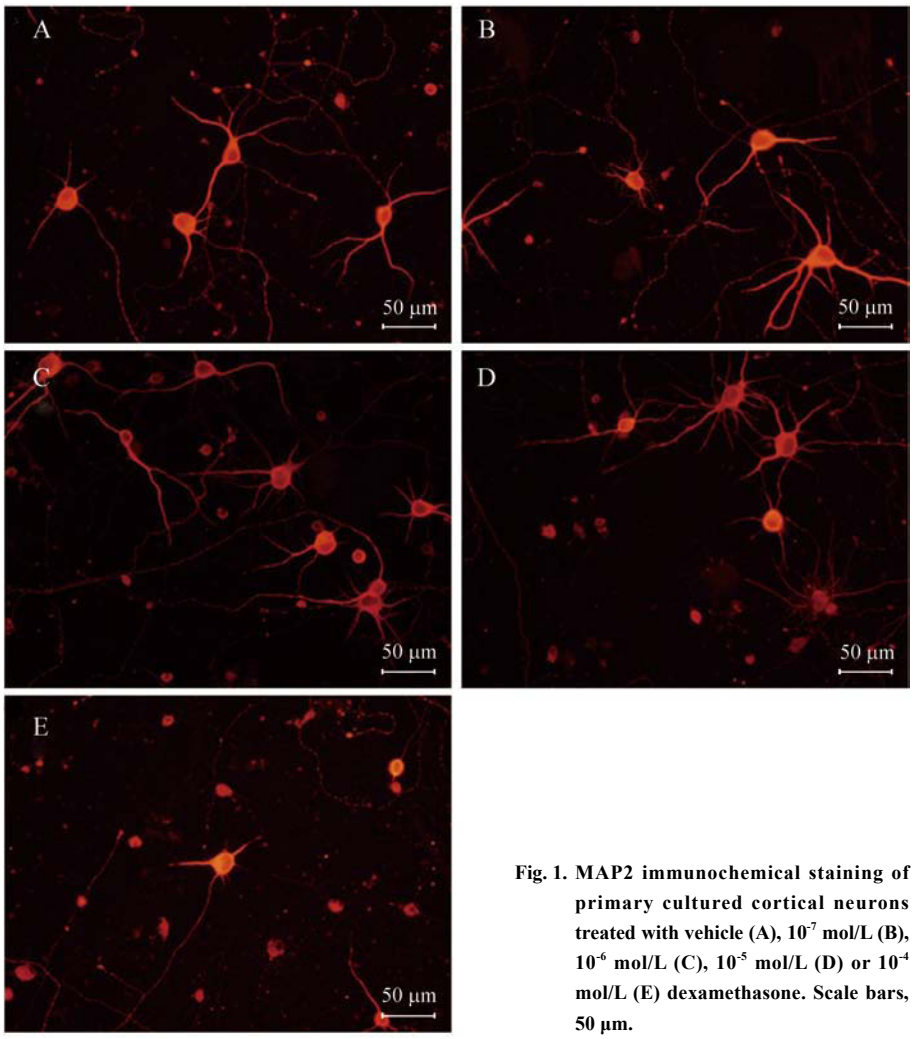

Fig. 1. MAP2 immunochemical staining of primary cultured cortical neurons treated with vehicle (A), 10^{-7} mol/L (B), 10^{-6} mol/L (C), 10^{-5} mol/L (D) or 10^{-4} mol/L (E) dexamethasone. Scale bars, 50 μm.

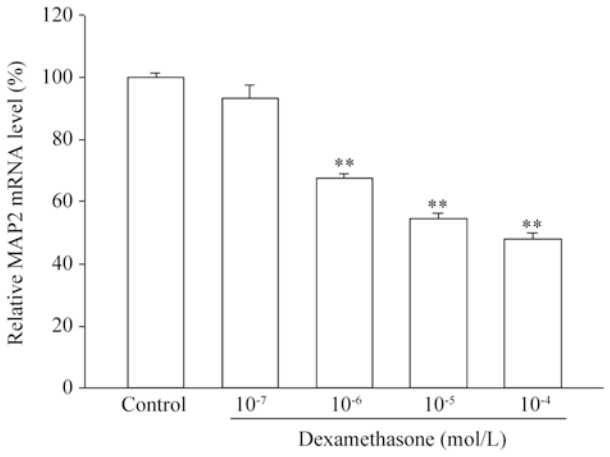

Fig. 2. Real-time PCR analysis of MAP2 mRNA levels in cortical neurons treated with dexamethasone (n = 3). **P <0.01 compared with control. Data were analyzed with one-way ANOVA followed by Bonferroni *post hoc* test.

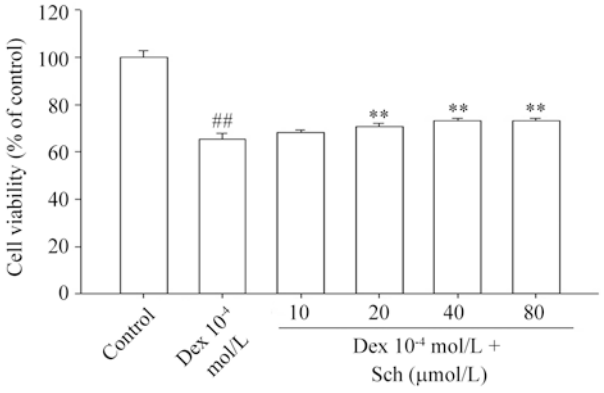

Fig. 3. Neuroprotective activity of schizandrin (Sch) against dexamethasone (Dex) neurotoxicity. Neurons were pretreated with 10^{-4} mol/L dexamethasone for 24 h, and then 10, 20, 40, or 80 μmol/L schizandrin for 48 h (n = 6). $^{\#\#}P$ <0.01 compared with control; **P <0.01 compared with dexamethasone 10^{-4} mol/L. Data were analyzed with one-way ANOVA followed by Bonferroni *post hoc* test.

in mice. The mean escape latencies and the percentage of swimming time in the platform quadrant were significantly different (Fig. 4, Tables S2–S3).

After administration of 15 or 45 mg/kg Sch for 14 days, the Dex-induced increase in escape latency and decrease in the percentage of swimming time in the platform quadrant were reversed (Fig. 4, Tables S2–S3). Moreover, compared with the chaotic and aimless track of mice with

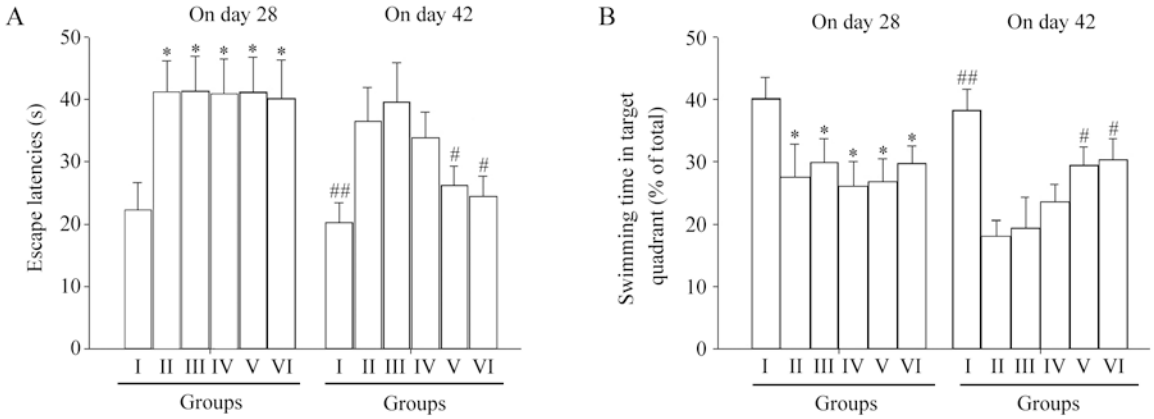

Fig. 4. Effects of dexamethasone and combined treatment with schizandrin (5, 15 and 45 mg/kg) on escape latencies (A) and percentage of swimming time in the platform quadrant (B) in the Morris water maze test. Values are mean ± SEM. n = 12. *P <0.05, **P <0.01 *vs* group I. $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 *vs* group II. Data were analyzed with one-way ANOVA followed by LSD *post hoc* test. Group I: control; group II: dexamethasone for 28 days; Groups III: dexamethasone for 42 days; Groups IV–VI: dexamethasone for 28 days followed by 5 (group IV), 15 (V), and 45 mg/kg·d^{-1} (VI) schizandrin (i.g.) for 14 days.

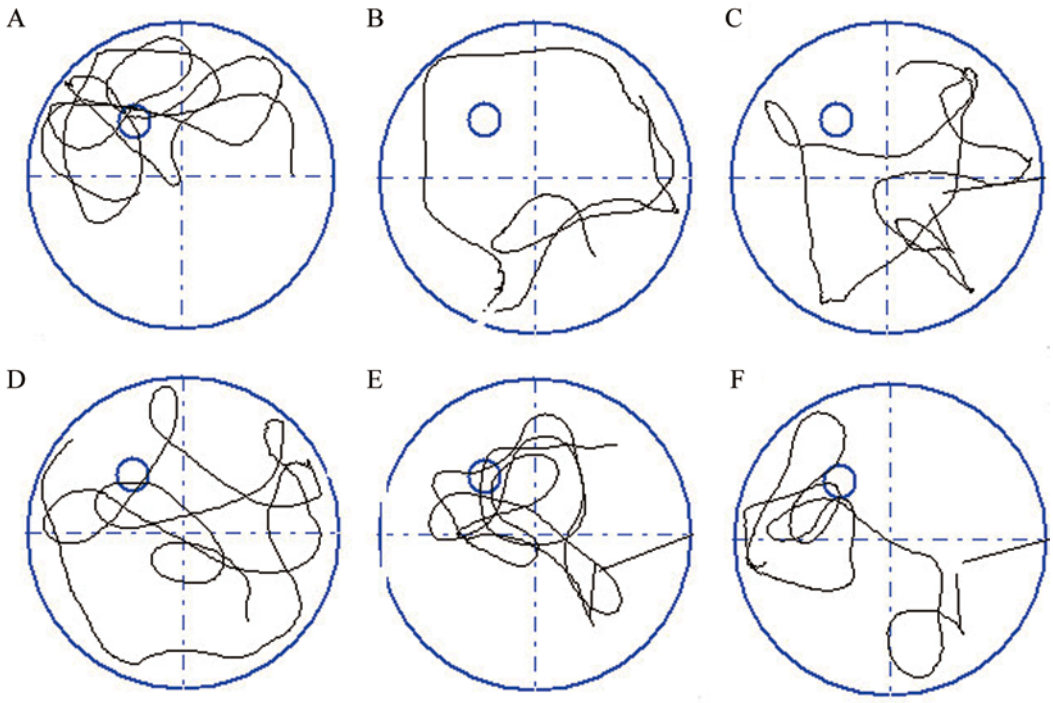

Fig. 5. Representative tracks of the final trials in the water maze test after the platform was removed. Mice were treated for 42 days. A: control; B: dexamethasone for 28 days; C: dexamethasone for 42 days; D–F: dexamethasone for 28 days followed by 5 (D), 15 (E), and 45 mg/kg·d^{-1} (F) schizandrin (i.g.) for 14 days.

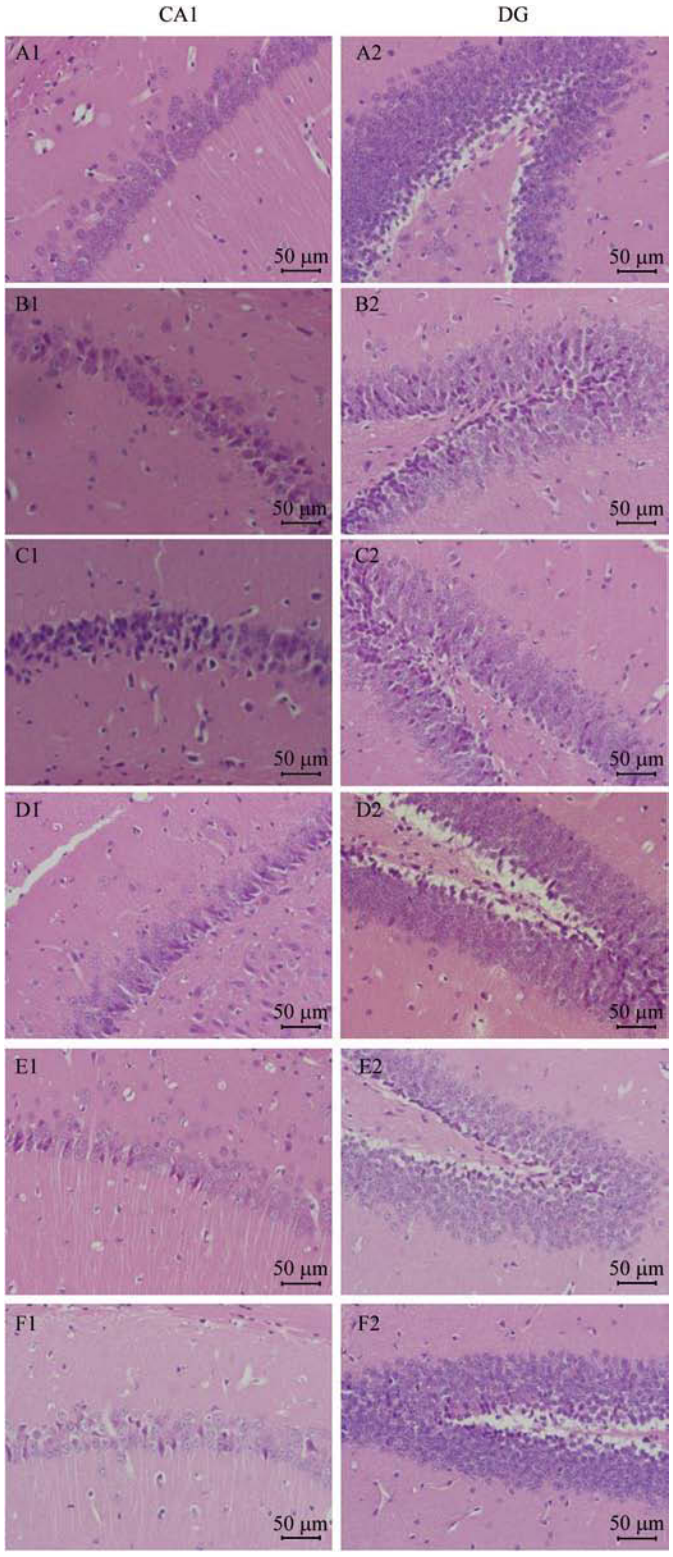

Fig. 6. HE staining of hippocampus of control mouse (A1–A2); mouse exposed to dexamethasone for 28 days (B1–B2), and for 42 days (C1–C2), dexamethasone for 28 days followed by 5 (D1–D2), 15 (E1–E2), and 45 mg/kg·d^{-1} (F1–F2) schizandrin for 14 days. Scale bars, 50 μm.

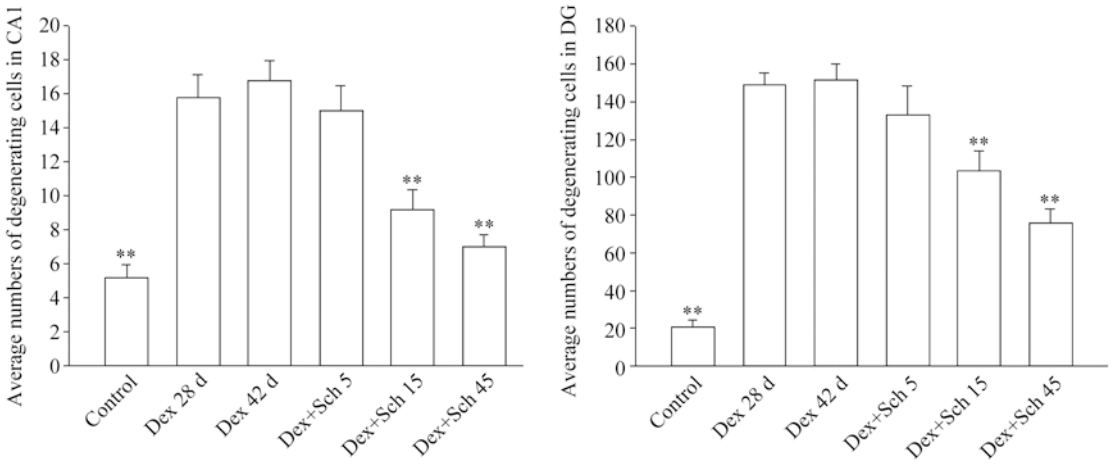

Fig. 7. Average numbers of degenerating cells in CA1 (left panel) and DG (right panel) that appeared smaller than normal and had condensed chromatin. **P <0.01 *vs* dexamethasone for 28 days (Dex 28 d). Data were analyzed with one-way ANOVA followed by LSD *post hoc* test. Dex 42 d, dexamethasone for 42 days; Dex + Sch 5/15/45, dexamethasone for 28 days followed by 5, 15, or 45 mg/kg·d^{-1} schizandrin for 14 days.

Dex treatment for 28 days, the 15 and 45 mg/kg Sch-treated groups were improved (Fig. 5). These results indicated that Sch significantly alleviates the cognitive impairment induced by Dex.

3.4 Effects of Sch on hippocampal structure in Dex-exposed mice No significant neuronal abnormalities were observed in the hippocampus (cornu ammonis I, CA1 and dentate gyrus, DG) in the control group, while the brains of Dex-treated mice showed neuronal degeneration. The somata of neurons became small and deeply stained with dye. But this morphological change was ameliorated by Sch; the average number of degenerating cells was lower in CA1 and DG, especially in the 15 and 45 mg/kg-treated groups (Figs. 6 and 7), Compared with that in Dex 28-day treatment group, corresponding with the performance in the Morris water maze test.

4 Discussion

Many studies in humans and animals have shown a close relationship between high GC levels and cognitive impairment, and Cushing's disease is associated with memory impairment[13,14]. In clinical practice, prolonged GC therapy may influence cognition[15-17], causing declarative memory impairment *via* cumulative and long-lasting detrimental influences on hippocampal function, including altered adrenal steroid receptor density, neurotransmitter content and dendritic atrophy[16,18,19]. Here, we found that Dex decreased neuronal viability and caused damage to neurites *in vitro,* as well as impairing spatial learning and memory *in vivo*. Our model can be used to screen for potential neuroprotective drugs against the neuronal impairment induced by GCs.

In vivo, it was reported that chronic (21 days) treatment with stress-levels of Dex induces learning and memory impairment in old male mice but not in young mice[20]. We also assessed the learning and memory in mice by the Morris water maze test after continuous treatment with Dex for 21 days, and obtained similar results (data not shown). So in our experiments, Dex administration *in vivo* was prolonged to 28 days, which successfully induced cognitive deficits in young KM mice.

Mice that received Dex for 28 days followed by vehicle for 14 days presented no significant differences in water maze performance, compared with mice receiving Dex for 42 days. These results suggested that the neuronal impairment induced by 28-day exposure to Dex was stable until the end of the experiment. This model thus provides a window of two weeks to assess the effects of potential drugs on cognition.

Chronic stress has been shown to alter the structure

and function of the hippocampus[21-23], and GC plays a central role in this process[24]. Stress and high GC levels cause reductions in hippocampal volume[25-27]. In addition, the hippocampus is not only a target for GCs but also a key structure for the feedback control of its secretion. Therefore, the death of hippocampal cells leads to failure of the feedback. This vicious cycle would lead to further neuronal loss. In the present study, we found morphological degeneration in the hippocampus from Dex-treated mice, which further confirmed that high levels of GCs cause severe brain damage.

Neuroprotective compounds with low molecular weight can readily cross the blood-brain barrier, and might have potential in the treatment of CNS disorders. In our studies, we screened neuroprotective compounds in Dex-exposed cortical neurons, and found that Sch protected neurons against a high level of Dex. The results *in vivo* further confirmed that Sch improved the spatial learning and memory, and alleviate hippocampal degeneration in Dex-treated mice. This is the first report that Sch prevents Dex-induced impairment *in vivo* and *in vitro*; however, the underlying mechanisms need to be further explored.

Supplemental Data: Supplemental Data include three tables and can be found online at http://www.neurosci.cn/epData.asp?id=33.

Acknowledgements: We thank Xing Zhou, Xiao-Wen Zhou, Zheng Zhang, Min-Jing Liao and Qin Gao for participation in the experiments. This work was supported by grants from the National Basic Research Development program (973 Program) of China (2011CB707500), the National Natural Science Foundation of China (81173037 and 30672450) and the Scientific program of Department of Science and Technology, Guangdong Province, China (2010B030700018).

References:

[1] Joels M. Corticosteroid effects in the brain: U-shape it. Trends Pharmacol Sci 2006, 27: 244–250.

[2] Joels M, Krugers HJ. LTP after stress: up or down? Neural Plast 2007, 2007: 93202.

[3] Roozendaal B. 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology 2000, 25: 213–238.

[4] Salehi B, Cordero MI, Sandi C. Learning under stress: the inverted-U-shape function revisited. Learn Mem 2010, 17: 522–530.

[5] Sandi C, Pinelo-Nava MT. Stress and memory: behavioral effects and neurobiological mechanisms. Neural Plast 2007, 2007: 78970.

[6] Michiels V, Cluydts R. Neuropsychological functioning in chronic fatigue syndrome: a review. Acta Psychiatr Scand 2001, 103: 84–93.

[7] Starkman MN, Giordani B, Berent S, Schork MA, Schteingart DE. Elevated cortisol levels in Cushing‘s disease are associated with cognitive decrements. Psychosom Med 2001, 63: 985–993.

[8] Kwon DY, Kim da S, Yang HJ, Park S. The lignan-rich fractions of Fructus Schisandrae improve insulin sensitivity via the PPAR-gamma pathways in *in vitro* and *in vivo* studies. J Ethnopharmacol 2011, 135: 455–462.

[9] Lin RD, Mao YW, Leu SJ, Huang CY, Lee MH. The immunoregulatory effects of *Schisandra chinensis* and its constituents on human monocytic leukemia cells. Molecules 2011, 16: 4836–4849.

[10] Lam PY, Leong PK, Chen N, Ko KM. Schisandrin B enhances the glutathione redox cycling and protects against oxidant injury in different types of cultured cells. Biofactors 2011, 37(6): 439–446.

[11] Stacchiotti A, Li Volti G, Lavazza A, Rezzani R, Rodella LF. Schisandrin B stimulates a cytoprotective response in rat liver exposed to mercuric chloride. Food Chem Toxicol 2009, 47: 2834–2840.

[12] Cheng HY, Hsieh MT, Wu CR, Tsai FH, Lu TC, Hsieh CC, *et al.* Schizandrin protects primary cultures of rat cortical cells from glutamate-induced excitotoxicity. J Pharmacol Sci 2008, 107: 21–31.

[13] Starkman MN, Gebarski SS, Berent S, Schteingart DE. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry 1992, 32: 756–765.

[14] Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, *et al.* Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nat Neurosci 1998, 1: 69–73.

[15] Brunner R, Schaefer D, Hess K, Parzer P, Resch F, Schwab S. Effect of corticosteroids on short-term and long-term memory. Neurology 2005, 64: 335–337.

[16] Brown ES, Woolston DJ, Frol A, Bobadilla L, Khan DA, Hanczyc M, *et al.* Hippocampal volume, spectroscopy, cognition, and mood in patients receiving corticosteroid therapy. Biol Psychiatry 2004, 55: 538–545.

[17] Bermond B, Surachno S, Lok A, ten Berge IJ, Plasmans B, Kox C, *et al.* Memory functions in prednisone-treated kidney transplant

patients. Clin Transplant 2005, 19: 512–517.

[18] Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000, 57: 925–935.

[19] Joels M. Corticosteroid actions in the hippocampus. J Neuroendocrinol 2001, 13: 657–669.

[20] Yao YY, Liu DM, Xu DF, Li WP. Memory and learning impairment induced by dexamethasone in senescent but not young mice. Eur J Pharmacol 2007, 574: 20-28.

[21] Kim JJ, Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002, 3: 453–462.

[22] Sapolsky RM. Chickens, eggs and hippocampal atrophy. Nat Neurosci 2002, 5: 1111–1113.

[23] Bremner JD. Stress and brain atrophy. CNS Neurol Disord Drug Targets 2006, 5: 503–512.

[24] McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol 2008, 583: 174–185.

[25] Sousa N, Lukoyanov NV, Madeira MD, Almeida OF, Paula-Barbosa MM. Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. Neuroscience 2000, 97: 253–266.

[26] Landfield PW, Blalock EM, Chen KC, Porter NM. A new glucocorticoid hypothesis of brain aging: implications for Alzheimer's disease. Curr Alzheimer Res 2007, 4: 205–212.

[27] Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 2009, 10: 434–445.