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Catalpol ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose

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Abstract

The neuroprotective effects of catalpol, an iridoid glycoside isolated from the fresh *Rehmannia* roots, on the senescent mice induced by D-galactose were assessed. The mice subcutaneously injected with catalpol (5 or 10 mg/kg, 2 weeks, from fifth week) showed significantly improved learning and memory ability in Morris water maze test compared with D-galactose treated mice (150 mg/kg, 6 weeks). We further investigated the mechanism involved in the neuroprotective effects of catalpol on the mice brain tissue. The results showed that catalpol increased the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), decreased the malondialdehyde (MDA) level, elevated the activities of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase on the cerebral cortex and hippocampus of D-galactose treated mouse. All the data suggested that catalpol had the potential to be a useful cognitive impairment treatment, and its beneficial effects may be partly mediated via enhancing endogenous antioxidant enzymatic activities and inhibiting free radical generation.

Keywords: Catalpol; Neuroprotection; Morris water maze; Learning and memory; Antioxidant

1. Introduction

Memory decline is characteristic of aging and age-related neurodegenerative disorders which lead to a progressive loss of cognitive function, especially in spatial memory (Barnes et al., 1980). It has been demonstrated that water maze learning and memory decline with increasing age of animals (Villarreal et al., 2002; Wu et al., 2002). Since the oxidative damage may play a role in the aging process, including the associated cognitive decline, age-related impairment in spatial learning and memory may be alleviated by antioxidant treatment (Carney et al., 1991; Socci et al., 1995).

Considerable evidence points to an important role of oxidative stress during the pathogenesis of age-associated or neurodegenerative diseases (Bowling and Beal, 1995; Markesbery, 1997; Perry et al., 2000). According to the free radical theory (Harman, 1956), the generation of reactive oxygen species (ROS) or free radicals can lead to cell and tissue damage paralleled by alterations in the function of the genetic apparatus, resulting in aging and untimely cell death. One approach to protect against ROS in aging and neurodegenerative disorders is to enhance oxidative defenses via antioxidants. Attention has been focused on a wide array of natural antioxidants that can scavenge free radicals and protect cells from oxidative damage, such as *Ginkgo biloba* extract (EGb 761) (Ilhan et al., 2006) and quercetin (Lu et al., 2006).

Rehmannia (known as Dihuang), which is far more frequently prescribed in China than in other countries, has been used to replenish vitality, strengthen the liver, kidney, and heart, and for treatment of a variety of ailments like diabetes, anemia, and urinary tract problems according to the Chinese Pharmacopoeia. Rehmanniae Decoction of Six Ingredients has long been used in age-related diseases and its therapeutic efficacy has been confirmed by many studies. Chronic administration of Rehmanniae Decoction of Six Ingredients to senescence-accelerated mouse promoted the spatial memory ability in water maze test and partially improved the learning behavior in conditioned avoidance performance (Zhou et al., 1999). Zhao et al reported that the contents of serum peroxide lipid, adrenocortical hormone, superoxide dismutase, plasmic testosterone, estradiol and cellular immune function in weak

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aged people had significant improvement after Liuwei Dihuang Pill treatment in clinical and pathological experimental study and Liuwei Dihuang pill indeed had the effect of anti-aging (Zhao et al., 2006).

There has been growing evidence that the extract from *Rehmannia* possesses significant neuroprotective activity (Cui et al., 2004; Yu et al., 2006), although little is yet known about the pharmacological effects or active ingredients. In our search for active ingredients, catalpol, an iridoid glycoside, was isolated from fresh root of *Rehmannia* with column chromatography method.

It has been reported that catalpol could protect PC12 cell from oxidative damage induced by H_2O_2 in vitro (Jiang et al., 2004) and exert neuroprotection effect on gerbils of transient global ischemia (Li et al., 2004a,b; Li et al., 2005). To our knowledge, there is no previous study on the anti-aging effects of catalpol in animal models of mice. Therefore, it is necessary to investigate the effect of catalpol on animal model for developing neuroprotective drug. Rodent chronically injected with D-galactose has been used as an animal aging model for brain aging or anti-aging pharmacology research (Wei et al., 2005). It was reported that D-galactose could impair neurogenesis in the dentate gyrus, a process similar to the natural aging in mice (Zhang et al., 2005). The aim of the present study was to investigate the effect of catalpol on the cognition of senescent mice induced by D-galactose and antioxidant/oxidant parameters in the cerebral cortex.

2. Materials and methods

2.1. Reagents and drugs

D-galactose was purchased from Shanghai Yuanju Chemical-Regent Company (Shanghai, China) and dissolved in 0.9% saline at concentrations of 3%. Catalpol (separation process see Section 2.2) was dissolved in physiological saline. Commercial kits used for determination of MDA, SOD, GSH-Px, Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase were purchased from Jiancheng Institute of Biotechnology (Nanjing, China). Plant material of *Rehmannia* was purchased from Yixian agriculture research institute, Liaoning province (China) and certified by Doctor Jiang B.

2.2. Preparation of Catalpol

Fresh root of *Rehmannia* (1 kg) were homogenated and extracted three times at room temperature with 95% EtOH (each 5 l) for 24 h. The extracts were filtered and evaporated under reduced pressure (50 °C, 0.08 MPa) to give a residue, which afforded a solution by dissolving in 1 l deionized H_2O. After centrifugation, the obtained solution was passed through a D101 macroporous resin column (the volume is 3 l) eluted with 9 l H_2O, 9 l 5% EtOH and 9 l 95% EtOH. The 5% EtOH elution on removal of the solvent under reduced pressure (50 °C, 0.08 MPa) provided 2.62 g brown powder, which was subjected to an open column chromatography on silica gel (100–140 mesh) eluted with a $CHCl_3$–MeOH gradient. The thin-layer chromatography (TLC) technique was used to detect the eluted materials from the column. Fraction (0.46 g) eluted with $CHCl_3$–MeOH (8:2) was identified as catalpol compared to its standard substance. In order to obtain pure catalpol, this fraction was subjected to another silica gel (200–300 mesh) column with same eluting solvent. The purity of the compound (0.28 g) was more than 90% purity by high-performance liquid chromatography (HPLC) analysis.

2.3. Animals and drug administration

The Kunming mice, obtained from Experimental Animal Center, Dalian Medical University, China. Animals, equal numbers of male and female, aged 3 months, weighing 25–30 g, were housed five each cage in an air-conditioned room with controlled temperature (25±2 °C) and maintained on a 12 h:12 h light cycle (07:00 on−19:00 off). They were allowed free access to food and water. The mice were randomly divided into three groups: control group (n=10), model group (n=10), and catalpol group (n=20). The mice of model group and catalpol group were subcutaneously injected with D-galactose at the dose of 150 mg/kg once daily for 6 weeks while those of control group were treated with same volume physiological saline. From the fifth week, catalpol group mice were subcutaneously injected with catalpol at the dose of 5 mg /kg or 10 mg/kg respectively after injection of D-galactose. Control group and model group mice were administered with same volume physiological saline. Behavioral testing was subsequently conducted between 9:00 and 17:00 for 7 days. Animals were sacrificed after behavioral tests for the biochemical assays. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Dalian Medical University, Dalian, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Each experimental protocol was statistically designed to use the minimal number of animals.

2.4. Behavioral testing

Behavioral testing was performed in the water maze (Morris, 1984), which consisted of a black circular tank, 100 cm in diameter and 50 cm in depth. The tank was divided virtually into four equal quadrants and an escape platform was hidden 1.5 cm below the surface of the water in a fixed location in the 3rd quadrant of the pool. After one day's training a trial was started by placing the mice into the pool close to the rim, facing the wall of the tank into one of the four quadrants. Mice were given four trials per session for 5 days, with each trial having a ceiling time of 60 s and a trial interval of approximately 30 s. After climbing onto the platform, the animal remained there for 30 s before the next trial. If the mice failed to reach the escape platform within 60 s, it was gently placed on the platform and allowed to remain there for 30 s. The time to reach the platform (latency) was measured. The day after the acquisition phase (5 days), a probe test was conducted by removing the platform. The time spent in the target quadrant, which had previously contained the hidden platform, was recorded. The latency to reach the non-exits and the numbers of crossing the non-exits were recorded for each trial.

2.5. Preparation of brain tissue and homogenates

After examination of the memory behavior, all mice were fasted overnight and then sacrificed by decapitation. The brains were removed. The cerebral cortex and hippocampus were dissected for the biochemical studies. Before detection, the cerebral cortex and hippocampus were homogenized in cold saline. The homogenate (10%) was centrifuged at 4000 ×*g* at 4 °C for 10 min, and the supernatant was used for assay.

2.6. Measurements of SOD activity, GSH-Px activity and MDA level in mice cerebral cortex and hippocampus

The assay for total SOD was based on its ability to inhibit the oxidation of oxymine by the xanthine–xanthine oxidase system (Oyanagui, 1984). The hydroxylamine nitrite produced by the oxidation of oxymine had an absorbance peak at 550 nm. One unit (U) of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%, and data were expressed as units per microgram of brain protein.

The activity of GSH-Px was determined by quantifying the rate of oxidation of the reduced glutathione to the oxidized glutathione by H_2O_2 catalyzed by GSH-Px. One unit (U) of GSH-Px was defined as the amount that reduced the level of GSH by 1 μmol l^{-1} in 1 min per milligram protein.

The level of lipid peroxidation in cerebral cortex homogenate was indicated by the content of MDA in brain tissue. Thiobarbituric acid reaction (TBAR) method was used to determine the MDA which can be measured at the wavelength of 532 nm by reacting with thiobarbituric acid (TBA) to form a stable chromophoric production (Ohkawa et al., 1979). MDA content was expressed as nanomoles per milligram of brain protein.

2.7. Determination of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities in mice cerebral cortex and hippocampus

ATPase activities were determined by using commercially available kits. All procedures completely complied with the manufacture's instructions. Theoretically, the activities of

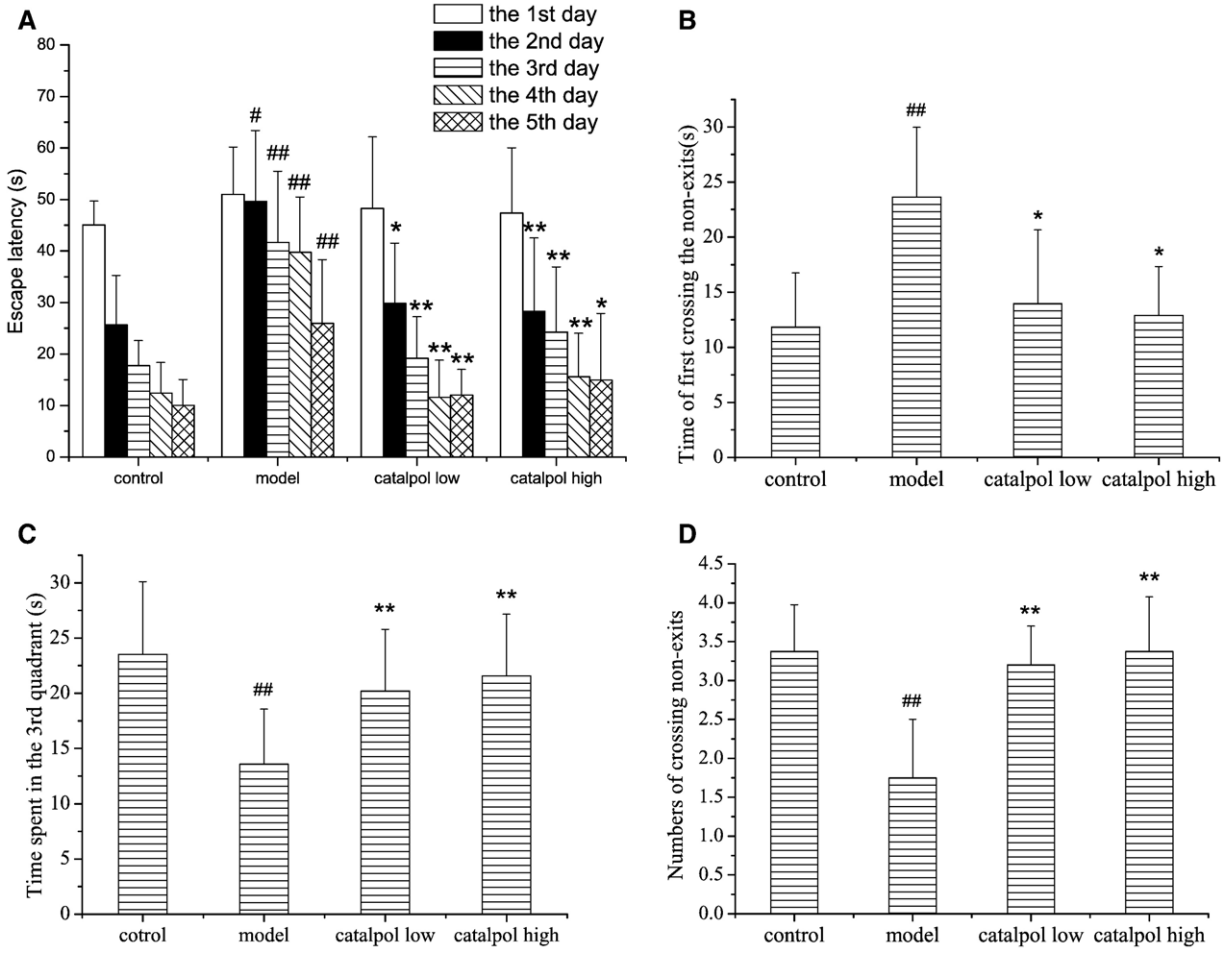

Fig. 1. Effects of catalpol on the spatial learning and memory of mice in the Morris water maze. (A) Escape latencies required by mice to find the hidden platform along 5 consecutive days. (B) The mean time of first crossing the non-exits. (C)The mean time spent in the target quadrant in which the platform had previously been located during acquisition. (D)The mean numbers of crossing non-exits. $^{\#}P<0.05$ vs. control group, $^{\#\#}P<0.01$ vs. control group; $^{*}P<0.05$ vs. model group, $^{**}P<0.01$ vs. model group. $n=10$.

ATPase were quantified by measuring the amount of inorganic phosphate (Pi) released by ATP hydrolysis according to the method of Heinonen and Lahti (Heinonen and Lahti, 1981). Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities were expressed as nmol Pi min^{-1} ml^{-1}.

2.8. Protein assay

Protein concentration was measured by the method of Bradford (Bradford, 1976). Bovine serum albumin was used as standard.

2.9. Statistical analysis

Statistical analysis was performed using OriginPro 7.5. All data in the text were expressed as mean±S.D., and analyzed by one-way ANOVA followed by a Student's *t* test. Linear regression analysis was performed to evaluate the correlations between two variables. A criterion of $P<0.05$ was accepted as statistically significant.

3. Results

3.1. Morris water maze test

The Morris water maze is a validated test used for the assessment of spatial learning and memory in mice. The results of the present study showed that the model group mice had significant cognitive deficits. As shown in Fig. 1A, the mean latency to find the platform declined progressively during the training days in all animals. However, the model group mice had longer latencies to platform throughout the training days than control mice ($P<0.01$), showing poorer learning performance due to chronic administration of D-galactose. Catalpol (5 or 10 mg/kg) treatment significantly shortened this prolongation of mean latency ($P<0.05$) as compared with the D-galactose treatment. On the probe trial, model group mice failed to remember the precise location of the platform, spending significantly much time to first crossing the non-exits than control and catalpol groups. (Fig. 1B; $P<0.01$ vs. control group, $P<0.05$ vs. catalpol group). The mean percent time spending in the target quadrant was increased by the administration of catalpol, (Fig. 1C; $P<0.01$ vs. model group) suggesting that catalpol, at least to some degree, reverses the memory deficits induced by D-galactose. Furthermore, the numbers of target crossing was significantly reduced in model group mice ($P<0.01$) pointing to a spatial navigation deficit. Catalpol treatment significantly reversed these spatial navigation deficits as seen in Fig. 1D ($P<0.01$ vs. model group). All

Table 1
Effects of catalpol on superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and lipid peroxides (MDA) in the cerebral cortex of senescent mice induced by D-galactose

Groups	SOD (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmol/mg protein)
Control group	95.21±10.34	2.56±0.45	3.94±0.76
Model group	57.08±8.49$^{\#\#}$	1.16±0.25$^{\#\#}$	8.77±0.98$^{\#\#}$
Catalpol low(5 mg/kg)	83.62±8.65**	1.73±0.18$^{\#}$**	4.70±1.34**
Catalpol high(10 mg/kg)	89.22±6.80**	1.76±0.32$^{\#}$**	4.04±1.18**

Values are expressed as mean ± SD. $^{\#\#}P<0.01$, $^{\#}P<0.05$ as compared to normol control; **$P<0.01$, *$P<0.05$ as compared to model group. $n=6$–8.

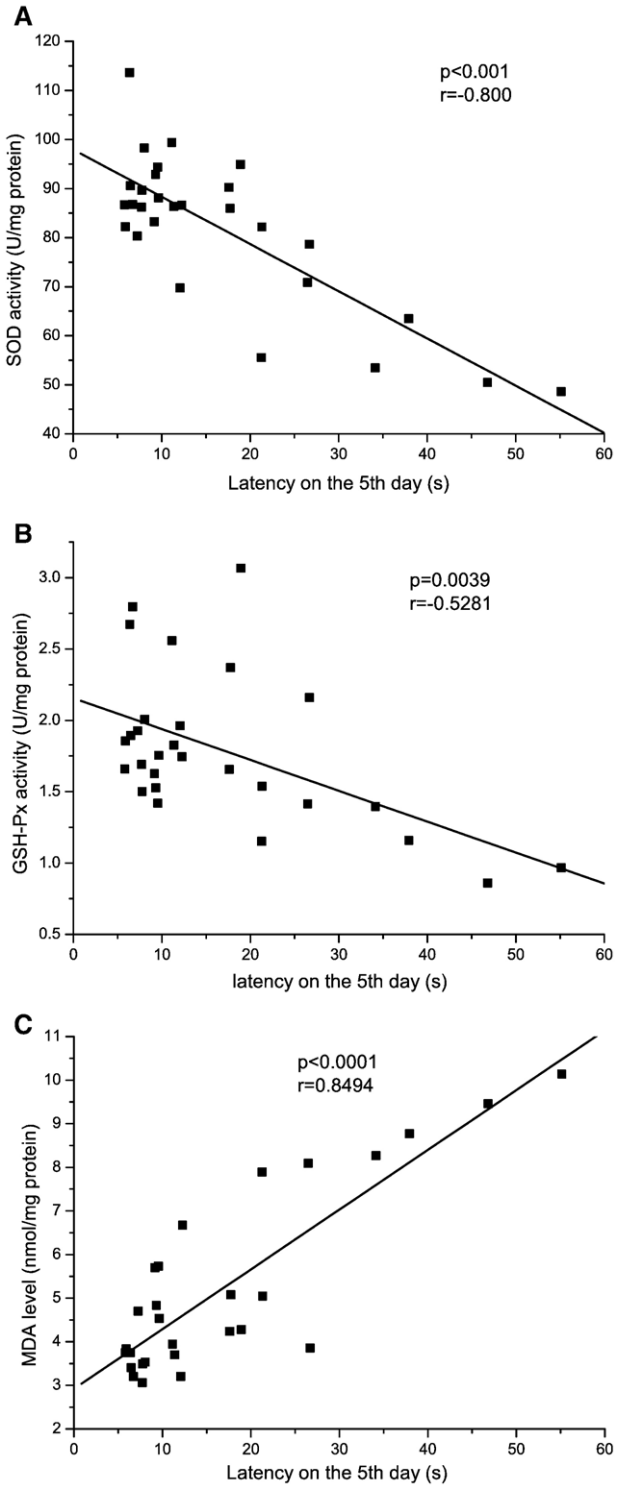

Fig. 2. Correlations between latency on the fifth day in the Morris water maze test and the activities of SOD (A), GSH-Px (B) and MDA level (C) in cerebral cortex of mice. The activities of SOD and GSH-Px were negatively correlated with learning deficits while the level of MDA was positively correlated.

results revealed that catalpol could improve the ability of spatial learning and memory in D-galactose treated mice.

3.2. Effects of catalpol on SOD, GSH-Px activities and MDA content in cerebral cortex of senescent mice induced by D-galactose

As compared with control group mice, the SOD activity in cerebral cortex significantly declined by 40.1% in model group mice and catalpol (5 or 10 mg/kg) could increase the activities of SOD nearly to 87.8%, 93.7% of the control group respectively (Table 1). All dose of catalpol reached significant levels ($P<0.01$) versus model group. There was no significantly difference between the control and the catalpol groups.

The activity of GSH-Px in cerebral cortex of model group mice was significantly lower as compared with control group (Table 1; $P<0.01$). Catalpol treatment resulted in a significant elevation in the activity of this enzyme. Higher dose of catalpol at 10 mg/kg ($P<0.01$ versus model group) had more significant effect than 5 mg/kg ($P<0.05$ versus model group). But there was still significantly difference between the control and the catalpol group ($P<0.05$).

Model group showed significant increase in MDA level compared with the control group (Table 1; $P<0.01$). This increase in MDA was also attenuated in the cerebral cortex of catalpol treated mice ($P<0.01$). There was no significantly difference between the control and the catalpol groups.

We further analyzed the correlation between latency on the fifth day of water maze test and the activities of SOD, GSH-Px and the level of MDA in the cerebral cortex of mice. As shown in Fig. 2A, latency to reach the platform on the fifth day was negatively correlated with the activities of SOD ($r=-0.800$, $P<0.001$). Such negative correlation between latency and GSH-Px activity also existed in the cerebral cortex (Fig. 2B; $r=-0.5281$, $P=0.0039$). The level of MDA was positively correlated with latency on the fifth day (Fig. 2C; r=0.8494, $P<0.0001$).

3.3. Effects of catalpol on SOD, GSH-Px activities and MDA content in hippocampus of senescent mice induced by D-galactose

The activities of SOD of model group mice were significantly lower as compared with control group ($P<0.01$), which were subsequently relieved by prolonged (2 weeks) catalpol administration (Table 2). Both doses of catalpol reached significant levels ($P<0.01$) versus model group. There was significantly difference between the control and the low dose catalpol groups ($P<0.05$) and not in high dose group.

The activities of GSH-Px in hippocampus significantly declined in model group mice as compared with control group

Table 2
Effects of catalpol on superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and lipid peroxides (MDA) in the hippocampus of senescent mice induced by D-galactose

Groups	SOD (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmol/mg protein)
Control group	60.25±3.98	1.81±0.08	2.32±0.54
Model group	41.20±2.54$^{\#\#}$	1.16±0.08$^{\#\#}$	4.50±0.28$^{\#\#}$
Catalpol low(5 mg/kg)	51.76±4.39$^{\#}$**	1.37±0.05$^{\#\#}$*	2.89±0.37**
Catalpol high(10 mg/kg)	55.41±5.52**	1.38±0.11$^{\#\#}$*	2.62±0.36**

Values are expressed as mean ± SD. $^{\#\#}P<0.01$, $^{\#}P<0.05$ as compared to normol control; **$P<0.01$, *$P<0.05$ as compared to model group. $n=6$–8.

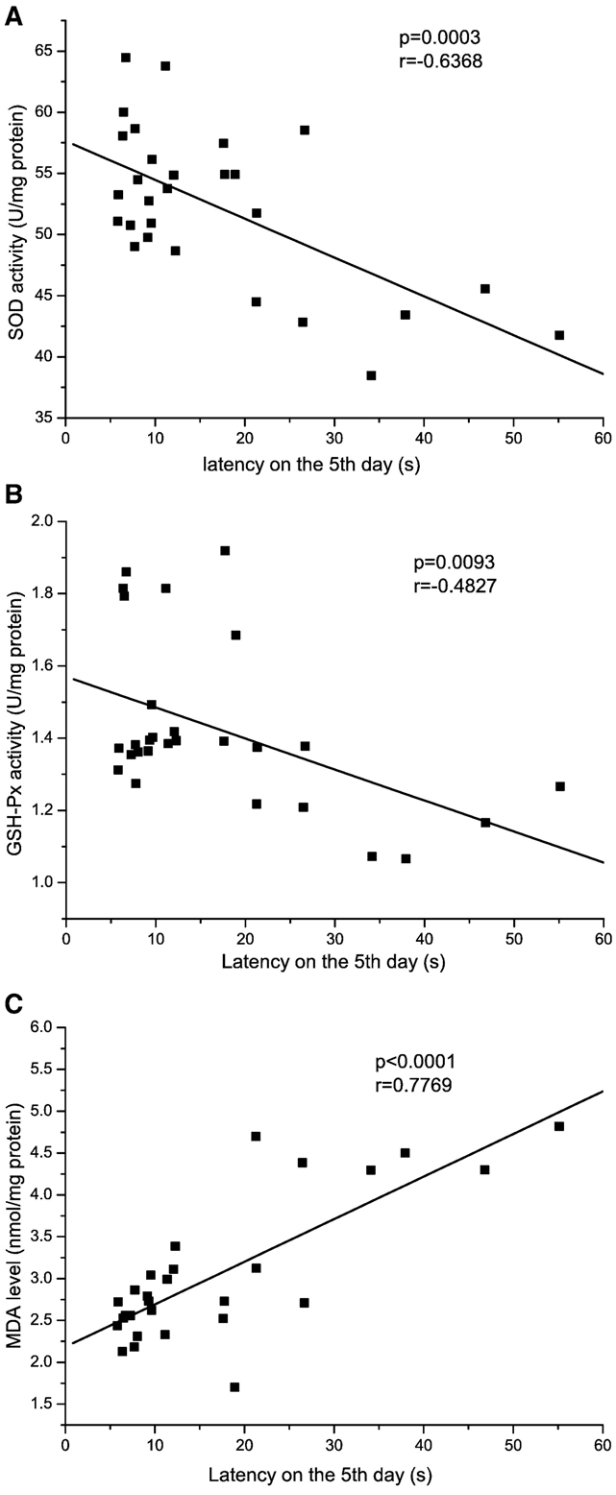

Fig. 3. Correlations between latency on the fifth day in the Morris water maze test and the activities of SOD (A), GSH-Px (B) and MDA level (C) in hippocampus of mice. The activities of SOD and GSH-Px were negatively correlated with learning deficits while the level of MDA was positively correlated.

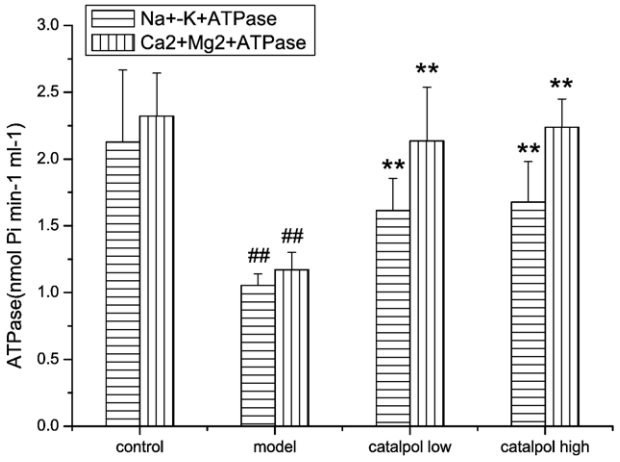

Fig. 4. Effects of catalpl on Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities in cerebral cortex of senescent mice induced by D-galactose. The activities of ATPases were decreased in model group mice ($^{\#\#}P<0.01$ vs. control group) and catalpol administration relieved this decrease ($^{**}P<0.01$ vs. model group). $n=6$–8.

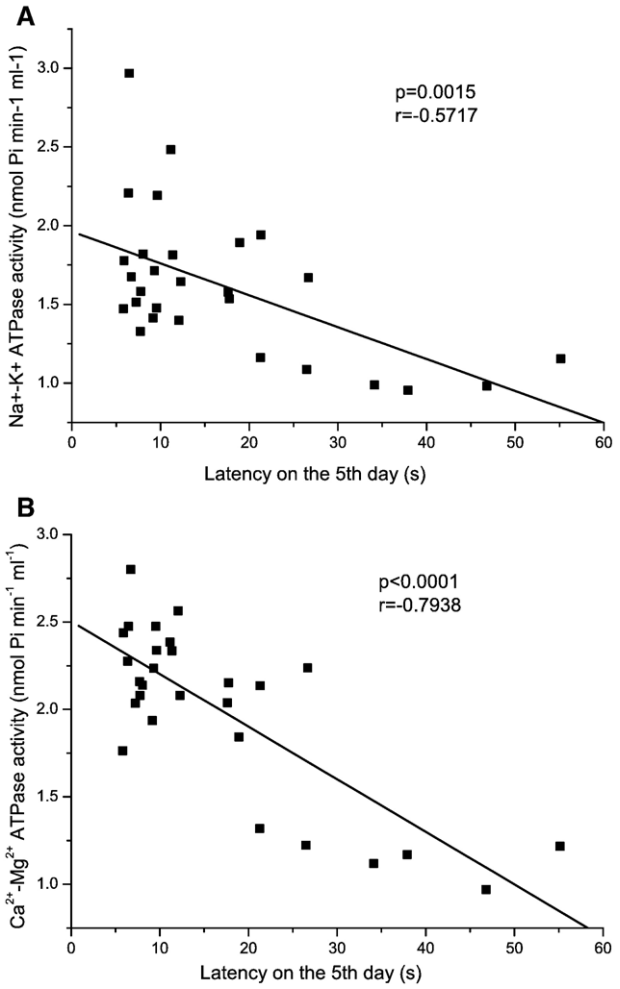

Fig. 5. Correlations between latency on the fifth day in the Morris water maze test and the activities of Na^+–K^+ ATPase (A) and Ca^{2+}–Mg^{2+} ATPase (B) in cerebral cortex of mice. The activities of ATPases were negatively correlated with learning deficits.

mice (Table 2; $P<0.01$). A significant enhancement of GSH-Px ($P<0.05$) activity was observed in response to catalpol at doses of 5, 10 mg/kg for 2 weeks as compared to model group. There was still significantly difference between the control and the catalpol groups ($P<0.01$).

The MDA level in hippocampus of model group was significantly higher than that of control group (Table 2; $P<0.01$) and this increase was attenuated by treatment with catalpol at doses of 5 or 10 mg/kg ($P<0.01$ vs. model group). There was no significantly difference between the control and the catalpol groups.

Furthermore, the SOD and GSH-Px activities in the hippocampus were negatively correlated with latency on the fifth day in the water maze test respectively ($r=-0.6368$, $P=0.0003$; $r=-0.4827$, $P=0.0093$) (Fig. 3A, B). The level of MDA was positively correlated with latency on the fifth day (Fig. 3C; $r=0.7769$, $P<0.0001$).

3.4. Effects of catalpol on Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities in cerebral cortex of senescent mice induced by D-galactose

Fig. 4 shows the changes of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities in cerebral cortex of senescent mice induced by D-galactose. It can be seen that model group mice had a significant decrease in Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities which were reduced by 49.6% and 50.4% respectively compared to control group in mice cerebral cortex. Catalpol treatment significantly protected the cerebral cortex against ATPase disturbances. The activities of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase found in catalpol group were higher than those in model group, corresponding to 75.9%, 91.9% of the control in low dose group and 78.9%, 96.4% of the control in high dose group respectively.

Moreover, the activity of Na^+–K^+ ATPase in the cerebral cortex were negatively correlated with latency on the fifth day

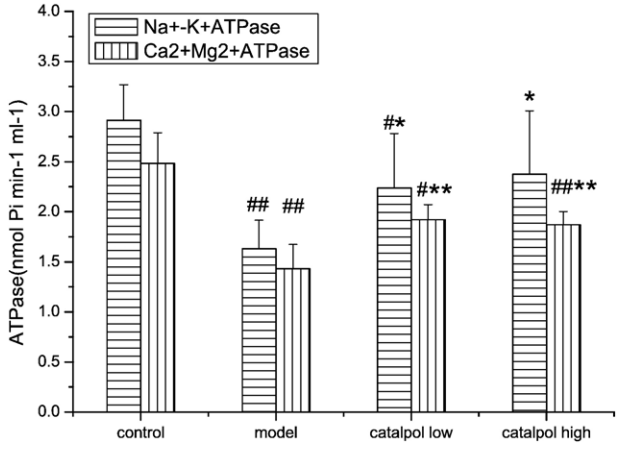

Fig. 6. Effects of catalpl on Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities in hippocampus of senescent mice induced by D-galactose. The activities of ATPases were decreased in model group mice ($^{\#\#}P<0.01$ vs. control group) and catalpol administration relieved this decrease ($^{*}P<0.05$ vs. model group). $n=6$–8.

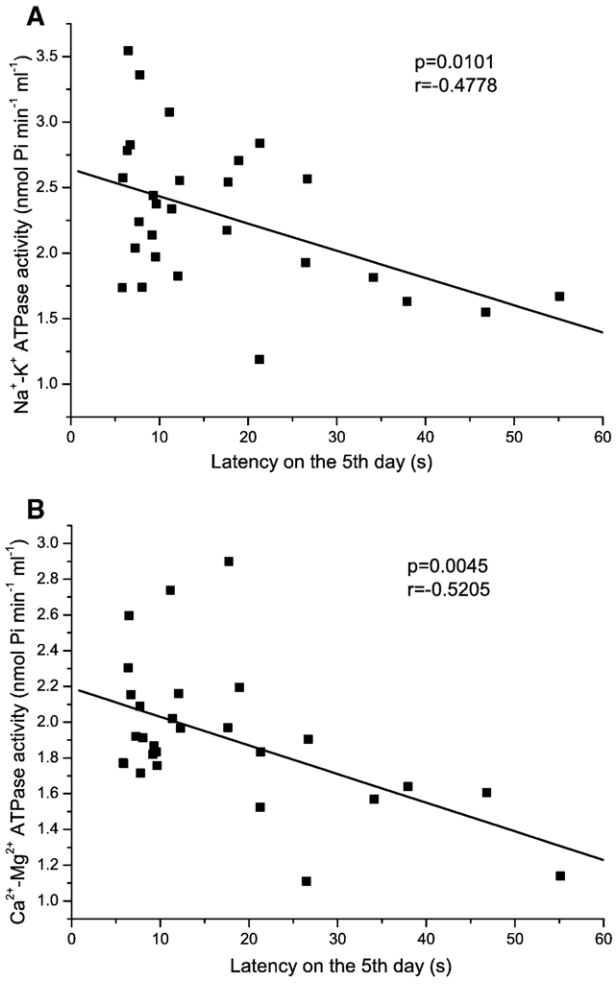

Fig. 7. Correlations between latency on the fifth day in the Morris water maze test and the activities of Na^+–K^+ ATPase (A) and Ca^{2+}–Mg^{2+} ATPase (B) in hippocampus of mice. The activities of ATPases were negatively correlated with learning deficits.

(Fig. 5A; $r=-0.5717$, $P=0.0015$). Such negative correlation also existed between latency and Ca^{2+}–Mg^{2+} ATPase activity (Fig. 5B; $r=-0.7938$, $P<0.0001$).

3.5. Effects of catalpol on Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities in hippocampus of senescent mice induced by D-galactose

As shown in Fig. 6, model group mice had a significant decrease in Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase activities which were reduced by 44.0% and 42.4% respectively compared to control group in mice hippocampus. The activities of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase found in catalpol group were higher than those in model group, corresponding to 76.9%, 77.4% of the control in low dose group and 81.4%, 75.4% of the control in high dose group respectively. And the activities of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase in the hippocampus were negatively correlated with latency on the fifth day respectively ($r=-0.4778$, $P=0.0101$; $r=-0.5205$, $P=0.0045$) (Fig. 7A, B).

4. Discussion

D-galactose is a reducing sugar that can form advanced glycation end product (AGE) in vivo, which can not be further metabolized and accumulates in nerve cells, at least partially contributing to the pathological mechanism of this aging model (Song et al., 1999; Tian et al., 2005). The free radicals generated from oxidation of D-galactose overrun the capacity of cells to clean them. This, consequently, causes the chain reaction of lipid peroxidation (LPO) and the end products, such as MDA, which combine with protein and phospholipid, lead to injury of cellular membrane and impairment of central nervous system (Hayakawa et al., 1992). The loss of membrane integrity leads to cellular dysfunction, such as loss of Ca^{2+} homeostasis, disruption of signal pathways and activation of nuclear transcription factors and apoptotic pathways. The neuronal death is the ultimate consequence of these cellular dysfunctions (Yatin et al., 1999). In the present study, subcutaneous administration of D-galactose for six weeks caused impairments in memory function and cognitive ability in mice. These results proved that D-galactose was suitable to use in the production of the senescent mice model.

ROS become an active field in aging research because of their potential involvement in many degenerative processes and in many neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Harman, 1992). These ROS can be scavenged by endogenous antioxidants including SOD and GSH-Px. MDA is a by-product of lipid peroxidation induced by free radicals and is widely used as a biomarker of oxidative stress (Cini et al., 1994). In this study, the activities of SOD and GSH-Px in the cerebral cortex and hippocampus both showed a statistically significant decline in model group mice compared to control group mice. Treatment with catalpol for two weeks could improve the activities of GSH-Px and SOD. In addition, an obvious enhancement of the level of MDA was shown in the model group mice, and it could be significantly reduced after catalpol administration. Therefore, catalpol scavenged ROS mainly via increasing the activity of SOD and GSH-Px, consequently, decreased lipid peroxidative damage.

It has been reported that the decreased activity of Na^+–K^+ ATPase is the consequence of either aging or peroxidative processes. Such effect might depend on the modification of the lipidic composition and decreased membrane fluidity, which occurs during aging (Viani et al., 1991). Catalpol significantly protected against Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase inactivation in cerebral cortex and hippocampus of senescent mice induced by D-galactose. It can be presumed that the elevation of Na^+–K^+ ATPase and Ca^{2+}–Mg^{2+} ATPase stabilize ions homeostasis, resulting a reduction in hydroxyl radical production in the brains of D-galactose treated animals, thus confirming the antioxidant action of catalpol.

In accordance with previous studies that natural iridoid compounds had neuritogenic effect on PC12 cells (Yamazaki et al., 1996) and lessened free radical-induced impairment of endothelium-dependent relaxation in rat aortic rings (Ismailoglu et al., 2002), it was demonstrated by our experiment that catalpol had ability to reduce oxidative stress induced by D-galactose in the way of activation of many antioxidant enzymes and ATPases.

Shen et al. (Shen et al., 2002) have reported that injection of D-galactose could induce senescent-like symptoms in animals, such as abnormal alterations in biochemistry markers, retrograde changes in neural cells and memory impairments. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice (Cui et al., 2006).Our results are in agreement with these findings. In our study, chronic administration of D-galactose impaired performance of mice in a water maze task and the catalpol group mice showed a shorter latency, indicating that catalpol had potential effects to prevent this kind of learning and memory deficits. Accumulating evidence (Fukui et al., 2002; Parle and Dhingra, 2003) has indicated that treatment with a variety of antioxidants partially reversed the increase in markers of oxidative stress and the decline in learning and memory.

We also found that there were correlations between the antioxidant parameters and cognitive parameters. Significant negative correlations were found between the latency to find the platform on the fifth day and the activities of SOD, GSH-Px and ATPases in mice cortex and hippocampus. The levels of MDA were positively correlated with the latency in the two regions of mice brain. These suggested that the oxidative damage may play a role in the cognitive decline of the senescent mice induced by D-galactose and catalpol against oxidative stress to brain may be involved in the mechanism of its action to ameliorate the impairments of learning and memory.

In conclusion, present findings indicated that chronic administration of D-galactose caused memory impairment and changes of some redox-related biomarkers in mice brain, including decrease in SOD, GSH-Px activities and increase of MDA level. Catalpol at the dose of 5 or 10 mg/kg significantly improved the cognitive impairment, increased the activities of endogenous antioxidant enzymes and relative ATPases in the brains of mice. Therefore catalpol may have potential as anti-aging therapy or in the treatment of neurodegenerative diseases.

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