ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12): 1149 - 1153
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Danggui Shaoyao San improve colchicine-induced learning acquisition impairment in rats

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KEY WORDS colchicine; learning disorders; avoidance learning; superoxide dismutase

ABSTRACT

AIM: To investigate the effects of the water extracts of Danggui Shaoyao San (DGSYS) in rats used the passive avoidance task. **METHODS**: Impairment in learning acquisition in rats was induced by colchicines, and the level of superoxide dismutase in the brain and the effects of DGSYS on the locomotor activity and pain threshold induced by colchicines were detected. **RESULTS**: DGSYS (0.1 - 1.0 g/kg) attenuated the impairment of learning acquisition induced by colchicine (15 μg) and DGSYS (0.5 and 1.0 g/kg) increased the level of SOD (141 ± 3 and 135.4 ± 2.0) in the brain. However, DGSYS (0.1 - 1.0 g/kg) did not affect the locomotor activity and pain threshold in the rats treated with colchicines. **CONCLUSION**: DGSYS can improve the learning acquisition deficit induced by colchicine in rats. The action mechanism of DGSYS may be involved in the increase in the level of superoxide dismutase.

INTRODUCTION

In general, memory processes are divided into three stages: *learning acquisition*, *memory consolidation*, and retrieval. According to biochemical studies, learning acquisition need the protein transport in cytoplasm, especially in signal transduction[1,2]. Colchicine is a mitosis inhibitor and proposes neurotoxicity in the binding to tubulin and disruption of axoplasmic transport[3,4]. In previously study, colchicine had been found that can induced learning acquisition deficits mainly via disturbing cortical choline acetyltransferase (ChAT) activity and decreaseing in the number of ChAT immunoreactive neurons in the nucleus basalis magnocellularis (NBM) after bilateral colchicine infusions for 5 weeks. Alzheimer's disease (AD) is a degenerative dementia that destroys the higher structures of the brain. People stricken with the disease face 10 to 1 years of deteriorating function, starting with memory impairment and ending with complete debilitation. Down-regulated ChAT activity is one of the hypotheses regarding the etiology of AD[5,6]. Establishing a valid animal model of the colchicine-induced learning acquisition is an impotent step in understanding the AD and exploring new treatments. On the other hand, Parkinson disease and AD are frequently associated with oxidative stress and defects in the cellular protective mechanisms. The lipid peroxidation (LPO) and the activity of the antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD) were evaluated in the hippocampus, striatum, and substantia nigra (SN). Superoxide dismutase (SOD) can be used as a therapeutic agent that can eliminate the superoxide anions, which play an important role in ischemia-reperfusion injuries[7-9]. An increase in the CAT and SOD activities in the hippocampus, striatum, and SN, and a decrease of the LPO in the hippocampus can be the indicators for ageing and impairment of learning and memory. Danggui Shaoyao San (DGSYS), is frequently described in the literature as *having sedative, anti-bacterial, blood-forming, and* immuno-enhancement properties to be used in treating various syndromes, especially for anemia and weakness[10,11]. DGSYS was modifying from Siwu Tang and were usually to treat the hemaopoietic and immunological disorder, hemasthenosis, and cerebral ischemia[12-14]. By contrast, the enhancement of the hemataboly may improve the impairment of learning and memory. In this present study, the step-through passive avoidance task was used to measure the learning acquisition stage depending on drug-treated period in colchicine-induced impairment to evaluate the mechanisms of the ameliorating drugs. In the study of oxidative stress, we attempted to investigate SOD activity in

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Received 2001-11-12 Accepted 2001-11-15

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

hippocampus to find out the probably mechanism[15].

MATERIALS AND METHODS

Experimental animal and lethal dose Male naive Sprague-Dawley rats, weighing 200 – 250 g, were given food and water *ad libitum* and kept in a regulated environment (23 ± 1 ℃), with a 12 h light-dark cycle (8:00 – 20 : 00, light). Each experimental group included 12 to 18 rats. The training trial with the corresponding method was done in the one week of the treatment. Male ICR, weighing 18 – 20 g, were used to detect the 50 % of lethal dose (LD_{50}) in which modified the method of Litchfield and Wilcoxon[16] and recorded the mice viability.

Preparation of DGSYS DGSYS was composed of seven medicinal plants as shown in Tab 1. DGSYS consists of crude ingredient extracted from 1.10 kg of herb medicine. Dr CHEN Chung-Chun, Institute of Chinese Pharmaceutical Science, made the botanical identification. Eleven liters of water circulated extract (50 ℃) for 4 h with 4 times re-extraction with 11 L of water every 4 h, then these supernatants were collected. This supernatant was centrifuged (5500 × *g*, 30 min) to remove insoluble ingredients, and concentrated the extraction to dry.

Tab 1. The ratio of the components in DGSYS.

Components	Ratio
1. Root of *Angelica sinensis* (Oliv) Diels	1
2. Root of *Paeonia lactiflora* Pall	4
3. *Poria cocos* (Schw) Wolf	1
4. Root of *Atractylodes macrocephala* Koidz	1
5. Root of *Alisma orientalis* (Sam) Juzep	2
6. Root of *Ligusticum chuanxiong* Hort	2

Colchicine induced learning impairment Colchicine and ampicillin were purchased from Sigma Chemical Co and dissolved in 0.9 % saline. There are 5 groups in this experiment including control, colchicine, colchicine with low (0.1 g/kg), medium (0.5 g/kg) and high (1.0 g/kg) dose of DGSYS. The rats were anaesthetized with 40 mg/kg of pentobarbital and a guide cannula equipped with a dummy probe was stereotaxically implanted above the right dorsal hippocampus. The stereotaxic coordinates of the guide tip were 4.2 mm posterior to bregma, 2.5 mm lateral to saggital suture, and 2.1 mm ventral to skull surface according to the Nakagawa *et al*[17]. Colchicine (15 μg) was microinjection to the hippocampus. Ampicillin (3.2 MU) was ip for 7 d and DGSYS (0, 0.5, and 1.0 g/kg) was oral administration for 14 d after surgical operation.

Step-through passive avoidance and behavior training trial The step-through passive avoidance task was used to measure the three-memory processes stage depending on drug-treated period[18,19]. This apparatus was consisted of two compartments having a steel-rod grid floor (36 parallel steel rods, 0.3 cm in diameter and 1.5 cm apart). One of the compartments (48 cm × 20 cm × 30 cm) was dark, and the other was equipped with a 20-W lamp located centrally at a height of 30 cm as the size, connected through a guillotine door (5 cm × 5 cm). The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00.

At the beginning of a training trial, the guillotine door connecting the light and dark compartment was closed. After each rat was placed in the light compartment with its back to the guillotine door, the door was opened and simultaneously the time (step-through latency, STL) taken by the rat enter to the dark compartment was measured with the stopwatch. Once the rat enters the dark compartment, the door was closed. An inescapable scrambled foot shock (1.0 mA, 2 s) was then delivered through the grid floor by MCU-101 Controller (Muromachi Likai Co, Tokyo). The rat was removed from the dark compartment 5 s after administering the shock. The rat was then put back to its home cage until the retention trial, which was carried out 24 h later. The rat was once again placed in the light compartment, and as in the case of the training trial, the guillotine door was opened and the STL was recorded and used as a measure of retention. An upper cutoff time of 300 s was set.

To evaluate the effect of various drug combinations on motor activity in passive avoidance task, the same experimental steps were followed as described in the above section with the exception that rat received no foot shock during the training period. Twenty-four hours later, the retention trial was also carried out and the STL was recorded. The rats were given 15 μg colchicine in combination with or without DGSYS for 14 d.

Pain threshold to electric stimulation The threshold of hoarsely squeak produced by electric shock was measured by using the passive avoidance apparatus. Each rat was shocked intensively and feet were then manually raised by stepwise increasing current from 0.6

to 1.0 mA with 0.1 mA interval until hoarsely squeak was observed. Duration of shock was 2 s, and the intershock interval was 5 s in each electric current (repeated for 3 times). The point at which continuous two times of hoarsely squeak was gauged as the pain threshold to electric stimulation. The rats were measurement after microinjection 15 μg of colchicine in hippocampus for 14 d, and detected 24 h later again.

Locomotor activity with nonshock rats To evaluated the effect of various drug combinations on locomotor activity in passive avoidance task by MK-ANIMEX activity meter (Columbus Co, USA). Record the times of each motor including work, stand up, arrangement hair, and recorded the each motor 24 h later again.

SOD activity determination The whole brain of rats was collected and homogenized in buffer at 4 ℃ that modified form Marklund and Marklund[20]. Briefly, brain tissue (0.5 g) was homogenized in 5 mL of sucrose-TE buffer (sucrose 0.32 mol/L, edetic acid 1 nmol/L, Tris-HCl 10 nmol/L, pH 7.4). Supernatant was collected after centrifuge (3600 × g, 4 ℃) for 30 min and diluted 50 times. The diluents combined with pyrogallol (final concentration: 200 μmol/L) in further 20 times diluting in Tris-HCl 5 mmol/L (pH 8.2). SOD activity was determinate by the spectrophotometer (420 nm) in each 40 s after mix well for 5 min. The automatic oxidation of pyrogallol was measure for 1 U when the 50 % of enzyme activity was inhibited in each time point. SOD activity was presented as U/mg of protein.

Statistics Because the data distribution from the passive avoidance task was truncated at 300, nonparametric Kruskal-Wallis analysis followed by two-tailed Mann-Whitney *U*-tests were used to analyze the data. The criterion for statistical significance was $P < 0.05$ in all the above statistical evaluations.

RESULTS

Colchicine (15 μg) injected immediately after the training trial significantly reduced the STL in the retention test. Mann-Whitney *U*-tests indicated that DGSYS (0.1, 0.5, and 1.0 g/kg, $P < 0.01$, Fig 1) significantly prevented colchicine-induced memory disruption. In locomotor activity counts, there were not different between with or without DGSYS (0.1 – 1.0 g/kg) administration (Fig 2). There were not different between colchicine with or without DGSYS in pain threshold to electric stimulation (Tab 2). In the SOD activity analysis, oral administrations of DGSYS (0.5 and 1.0 g/kg) can significantly enhance the SOD activity in brain ($P < 0.01$, Tab 3). The LD_{50} of DGSYS is more than 10 g/kg.

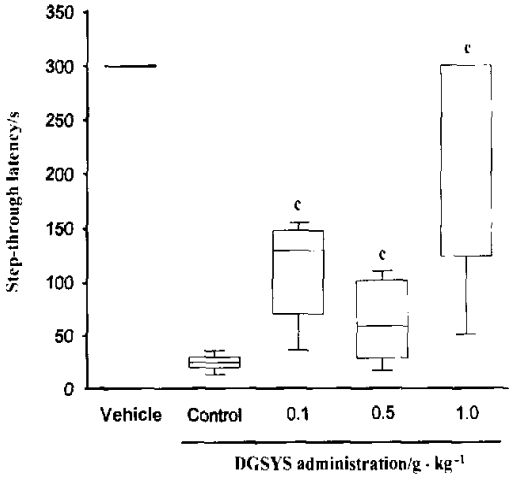

Fig 1. Preventive effect of DGSYS after two weeks consecutive administration from the colchicine induced learning acquisition impairment of passive avoidance response in rats. Each column and centerline in the column represents the 95 % confidence interval and the median. $n = 6$. $^cP < 0.01$ *vs* Vehicle group.

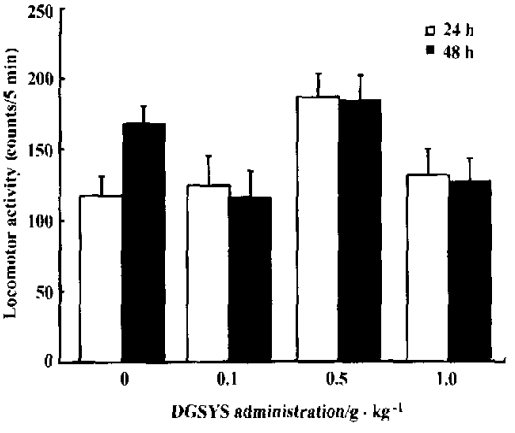

Fig 2. Effects of DGSYS administrated for two weeks on the locomotor activity produced by colchicine-induced impairment in rats. $n = 6$. $\bar{x} \pm s_{\bar{x}}$.

DISCUSSION

The LD_{50} of DGSYS is more than 10 g/kg, which

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
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Tab 2. Threshold of vocalization in colchicines and combined with or without DGSYS in rats. $n=6$. $\bar{x} \pm s_{\bar{x}}$.

Treatment	Threshold (mA) of vocalization
Control	0.84 ± 0.02
Colchicine (15 μg)	0.82 ± 0.04
Colchicine + DQSYS (0.1 g/kg)	0.80 ± 0.01
Colchicine + DQSYS (0.5 g/kg)	0.87 ± 0.03
Colchicine + DQSYS (1.0 g/kg)	0.84 ± 0.01

Tab 3. Effects of DGSYS on the level of SOD induced by colchicine in the brain of rats. $n=6$. $\bar{x} \pm s_{\bar{x}}$. $^{c}P<0.01$ ***vs* Colchicine group (One-way ANOVA following by Scheffe's test).**

Treatment	Level of SOD (U)
Control	145 ± 3
Colchicine (15 μg)	89.6 ± 1.7
Colchicine + DQSYS (0.5 g/kg)	141 ± 3^{c}
Colchicine + DQSYS (1.0 g/kg)	135.4 ± 2.0^{c}

means DGSYS is more safety in toxicity. Colchicine-induced impairment of passive avoidance response had been found to act through the disturbance[21,22]. Colchicine binds to tubulin and is toxic to certain populations of neurons in the brain, especially those of the hippocampalformation. Several reports indicate that direct injection of colchicine into hippocampus preferentially destroys granule cells in the dentate gyrus[23,24]. In this present study, colchicine can significantly reduce the learning acquisition in our model. The further application in the DGSYS was used in arranging the defects that colchicines-induced learning impairment. In the prevention of the oxidative stress, SOD activities were determined by reduced the pyrogallol anti-oxidation[25]. Administrations of DGSYS (0.5 and 1.0 g/kg for 14 d) can significantly recovery the SOD activity that impaired by colchicine. On the other hand, the pain threshold to electric stimulation and locomotor activity counts showed that DGSYS recover colchicines-induced impairment not by analgesia and calm. Taking all these observations into account, we proposed that DGSYS prevented the rats from colchicine-induced learning impairment. The ameliorating mechanisms of DGSYS on colchicine-induced learning impairment may be via enhancing the SOD activity and reducing the oxidative stress. The further application of this medicine might be used in treatment the AD or Parkinson disease by recovering the SOD activity.

ACKNOWLEDGEMENTS I was grateful to Dr Weng-Huang PENG, Mr Chi-Ming HO, and Mr Chang-Chi HSIEH for their excellent technical assistance.

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当归芍药散改善秋水仙碱诱导的大鼠记忆获得障碍

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关键词: 秋水仙碱; 学习障碍; 回避学习; 超氧化物歧化酶

目的: 本研究探讨当归芍药散水提取物对秋水仙碱诱导的被动回避学习障碍的影响及脑中超氧化物歧化酶含量的变化. **方法**: 以电痛刺激实验及大鼠自发运动量仪测定大鼠被动回避学习、痛阈值及运动量改变. **结果**: 当归芍药散水提取物(0.1 - 1.0 g/kg)可明显改善秋水仙碱诱导大鼠之被动回避学习获得障碍, 并增加秋水仙碱诱发的大鼠脑中超氧化物歧化酶的含量. 但对痛阈值和运动量并无影响. **结论**: 当归芍药散水提取物对秋水仙碱诱导的被动回避学习获得障碍有改善作用, 其作用可能与改善秋水仙碱造成的细胞损伤或增加脑中超氧化物歧化酶含量有关, 而非与镇痛和镇静有关.

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