ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12): 1078 - 1083
http://www.chinaphar.com

Original Research

Improving effects of SSF on memory deficits and pathological changes of neural and immunological systems in senescent mice

SHANG Ya-Zhen[1], GONG Ming-Yu, ZHOU Xiao-Xia, LI Su-Ting, WANG Bao-Yuan
(*Institute of Materia Medica, Chengde Medical College, Chengde 067000, China*)

KEY WORDS *Scutellaria baicalensis* George; memory disorders; maze learing; galactose; neuroimmunomodulation

ABSTRACT

AIM: To study the effects of SSF, an effective part isolated from *Scutellaria baicalensis* George, on memory impairments and the pathological changes of neural and immunological systems in senescent mice induced by chronic galactose. **METHODS**: Senescent performance in mice was induced by consecutive administration of *D*-galactose (120 mg/kg, ip) for 47 d. The behavioral tests of mice used water maze task. The neural and immunological changes were assessed by alterations of cerebral cortex morphology and immune tissue index. The improving effects of SSF (50, 100, and 200 mg/kg, ig, 47 d) on above changes in the senescent mice were detected. Piracetam (PIR) was as reference drug. **RESULTS**: *D*-Galactose (120 mg/kg, ip, 47 d) resulted in an increase in the latencies to find the terminal platform and the number of errors entering non-exits in water maze, neuropathological changes and immune tissue index (spleen index) deducted in mice as compared with saline treated group. Both PIR (200 mg/kg, ig, 47 d) and SSF (50, 100, and 200 mg/kg, ig, 47 d) could significantly reverse the increased latencies and number of errors and improve the pathological alterations of neural and immunological systems. **CONCLUSION**: SSF could ameliorate the cognitive deficits and pathological alterations of neuron and immune systems in senescent mice induced by chronic galactose.

[1] Correspondence to Assoc Prof SHANG Ya-Zhen.
Phn 86-314-206-9594. Fax 86-314-206-9594.
Received 2001-06-17 Accepted 2001-10-19

INTRODUCTION

Brain senescence played an important role in aging tissues. The main clinical performances of brain senescence were cognitive deficits and tardiness in responses. Its chiefly pathological performance was decreased in neuronal synapse, showing neurofibrillary tangles as well as significant neural cell loss[1].

Senescence in humans was accompanied by impairments in learning memory[2]. The impaired memory in senescent animals was also found in various tests of learning and memory such as step down tasks[3,4], passive avoidance tasks[5,6], and water maze tasks[7,8]. Many papers have reported that degeneration in neural system may contribute to senescence-related memory impairments even some immunological functions[9,10]. There was evidence showing that galactose could result in similar senescent performances in animals to aging humans such as abnormal data in biochemistry, degeneration in immunological activities, controlling abnormal in gene expression, deduction in propagating ability, retrograde changes in neural cells, and memory impairments. These produced senescent mechanisms might be correlated with lipid peroxidation[11-14].

SSF, an effective part isolated from stems and leaves of *Scutellaria baicalensis* George, had very stable physical nature and many hydroxyls special structure, the structure made it have many pharmacological effects. We have reported that SSF had significant anti-hyperxia[15] and ameliorated impaired memory caused by some chemical regents in mice (in preparation). The aim of this study was to investigate the effects of SSF on cognitive deficits and neuropathology and immune changes in mice induced by galactose.

MATERIALS AND METHODS

Mice Kunming mice (♀, 22 - 24 g, Clean

grade, Certification № 04056) were supplied by the Experimental Animal Center, Chengde Medical College. Mice were group-housed (ten mice per cage), and were maintained in a climatically controlled room on a 12∶12 h light∶dark cycle with free access to food and water.

Water maze All mice were trained in mice water maze. The latencies to find the terminal platform, and number of errors entering non-exits in the maze were used to evaluate performances. The water maze apparatus was a double-layer opaque plastic box (80 cm × 50 cm × 20 cm) including a start point, a terminal platform, and four non-exits. Near the platform was the safe region and a light was located as a mark. The maze was filled with water to depth of 12 cm and the temperature was kept (20 ± 1) ℃. Every spatial sign around the maze was kept fixed for the whole test. On the first day of mice training, each mouse was allowed to swim in the maze to be accustomed to swimming for 15 min. From the second day, the mice were trained to find the terminal platform. When the mice arrived the terminal platform within 60 s, they were allowed to remain there for 20 s. If the mice did not find the platform within 60 s, they were driven to the platform with help and remained on it for 20 s. Each mouse received 2 trials per day for 5 consecutive days. The data were calculated for an average of 2 trials a day.

Drug administration SSF was provided by Department of Phytochemistry in this Institute. Piracetam was purchased from Tianjin Jinshi Pharmaceutical Co. *D*-Galactose was from Beijing Chemical-regent Co. Other regents were AR grade. These compounds were dissolved in distilled water prior to administration and PH of SSF solution was made at 7.2 – 7.4 with sodium bicarbonate. Galactose (120 mg/kg) or saline was injected ip, simultaneously, SSF (50, 100 and 200 mg/kg), PIR (200 mg/kg), or saline administered ig for 42 d (once a day). From d 43, the mice were administered ig SSF or PIR or saline 60 min and ip galactose or saline 30 min before being daily trained the water maze performance. The administration volume was kept constant at 0.2 mL per 10 g irrespective of dose.

Neuromorphology and immunological system Three to four mice chosen randomly from each group were decapitated after the behavior experiment. The brain was rapidly dissected on ice. The cerebral cortex was separated and routinely processed and embedded in paraffin for histopathological observation. Coronal sections were stained with hematoxylin and eosin (HE). The body, spleen, and thymus weight of each mouse in every group was measured and the immune index was evaluated. The levels of immune index were calculated as follows:

Immune index (mg/g) = W_1/W_2, Where W_1 and W_2 were weight of immunological tissue (thymus or spleen) and body, respectively.

Statistical analysis All results were expressed as $\bar{x} \pm s$. The behavior test data used ANOVA followed by Duncan's multiple-range test, Others were compared by Student's t-test.

RESULTS

Effects of SSF on impaired memory in senescent mice induced by galactose Spatial memory of mice treated with saline, galactose, or PIR and galactose, or SSF and galactose was tested in the water maze. During 5 d water maze trials, the galactose group mice consistently took longer latencies to find the terminal platform than saline group and the effect was statistically significant ($P < 0.05$). The prolonged latencies in galactose mice were shortened by SSF at dose of 50, 100, and 200 mg/kg and the effects showed in a dose-dependent manner. The results which three dose of SSF significantly lessened the latencies and the scores of SSF 200 mg/kg mice were much better than saline treatment in Fig 1A were parallel to Fig 1B. The same results were observed in number of errors. PIR (200 mg/kg) was also revealed improving effects on senescent mice caused by galactose in the water maze task (Fig 1, 2).

Effects of SSF on neuropathological changes in senescent mice induced by galactose Fig 3 showed that the typical neuropathological changes were observed on cerebral cortex in senescent mice administered galactose for 47 d. Compared with the saline group, round neuron, shrinkage, neurofibillary degeneration, and dark staining of neuron were observed in cerebral cortex in galactose treated mice. Consecutive administration of SSF (50, 100, and 200 mg/kg) could significantly attenuate these neuropathological changes. The neural cell became full and was shaped variously. Prolonged neurofibillary and light staining of neuron were revealed. The results in SSF 200 mg/kg mice were much better than treated saline mice. The potent results were also observed in galactose mice with PIR 200 mg/kg.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
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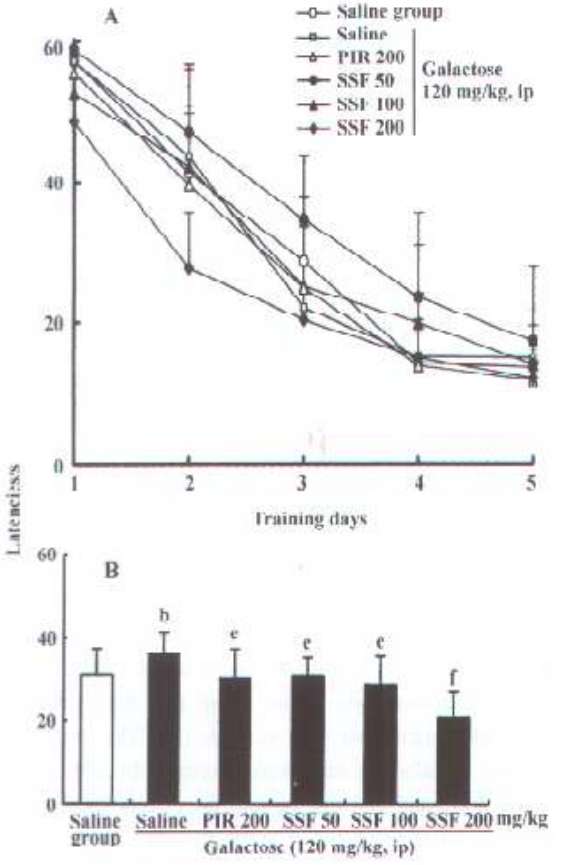

Fig 1. Effects of SSF (50, 100, and 200 mg/kg) and PIR (200 mg/kg) on mice water maze performance deficits caused by galactose in mice. A: mean escape latencies to find the terminal platform was shown. B: the mean escape latencies for 5 days. $n = 10$. $\bar{x} \pm s$. $^bP < 0.05$ *vs* saline-treated group. $^cP < 0.05$, $^fP < 0.01$ *vs* galactose-treated group.

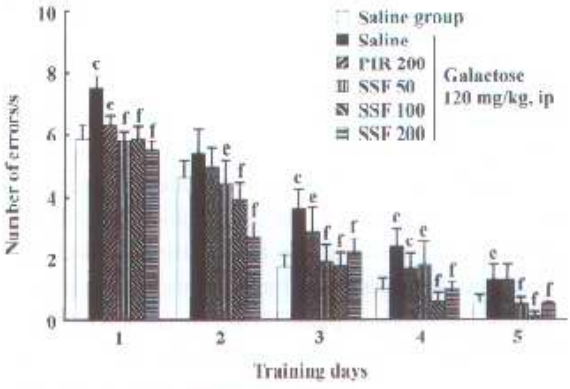

Fig 2. Effects of SSF (50, 100, and 200 mg/kg) and PIR (200 mg/kg) on mice memory deficits induced by galactose in water maze task. The mean number of entering non-exits was shown. $n = 10$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* saline-treated group. $^cP < 0.05$, $^fP < 0.01$ *vs* galactose-treated group.

Tab 1. Effects of SSF on spleen weight in mice after consecutive ip galactose for 47 d. $n = 10$ mice. $\bar{x} \pm s$. Data were expressed as spleen index (mg/g body weight). $^bP < 0.05$ *vs* saline group. $^cP < 0.05$, $^fP < 0.01$ *vs* galactose group.

Group	Dose/mg·kg^{-1} (qd × d)	Spleen index
Saline group	–	6.32 ± 2.66
Galactose group	–	4.40 ± 1.13^b
PIR	200 × 47	5.21 ± 1.72
SSF	50 × 47	5.89 ± 1.79^c
	100 × 47	5.93 ± 1.98^c
	200 × 47	7.59 ± 1.18^f

Effects of SSF on immunological changes in senescent mice induced by galactose Compared with saline group, galactose (120 mg/kg, ip, 47 d) produced a decrease in mice spleen weight and the spleen index reduced 30.38 % ($P < 0.05$). Consecutive administration of SSF significantly deduced the decrease. SSF increased the spleen index of galactose mice by 33.86 % (50 mg/kg), 34.77 % (100 mg/kg), and 72.5 % (200 mg/kg), respectively, as compared with galactose treatment. However, no marked changes were observed in thymus weight in all mice and these effects of PIR were not significant (Tab 1).

DISCUSSION

Among the animal models which have been developed to study humans dementia, long-term administration of galactose induced amnesia in rodents based on their cognitive deficits accompanied by progressive neuronal damage such as neuron loss, neuron transcription ability decrease, RNA concentration deduction in brain tissue[16]. SOD and GSH-Px activities declination, and MDA and lipofuscin lever increase. These alterations in brain were considered to play important roles in learning and memory[11-13,17]. In the present behavioral trials, learning and memory performance in water maze task was severely impaired in administered galactose mice. The results agreed well with previous reports that brain senescence resulted in an increase in the time required to find the terminal platform and number of error entering the non-exits[18]. We also found that chronic galactose induced neural cell damage in

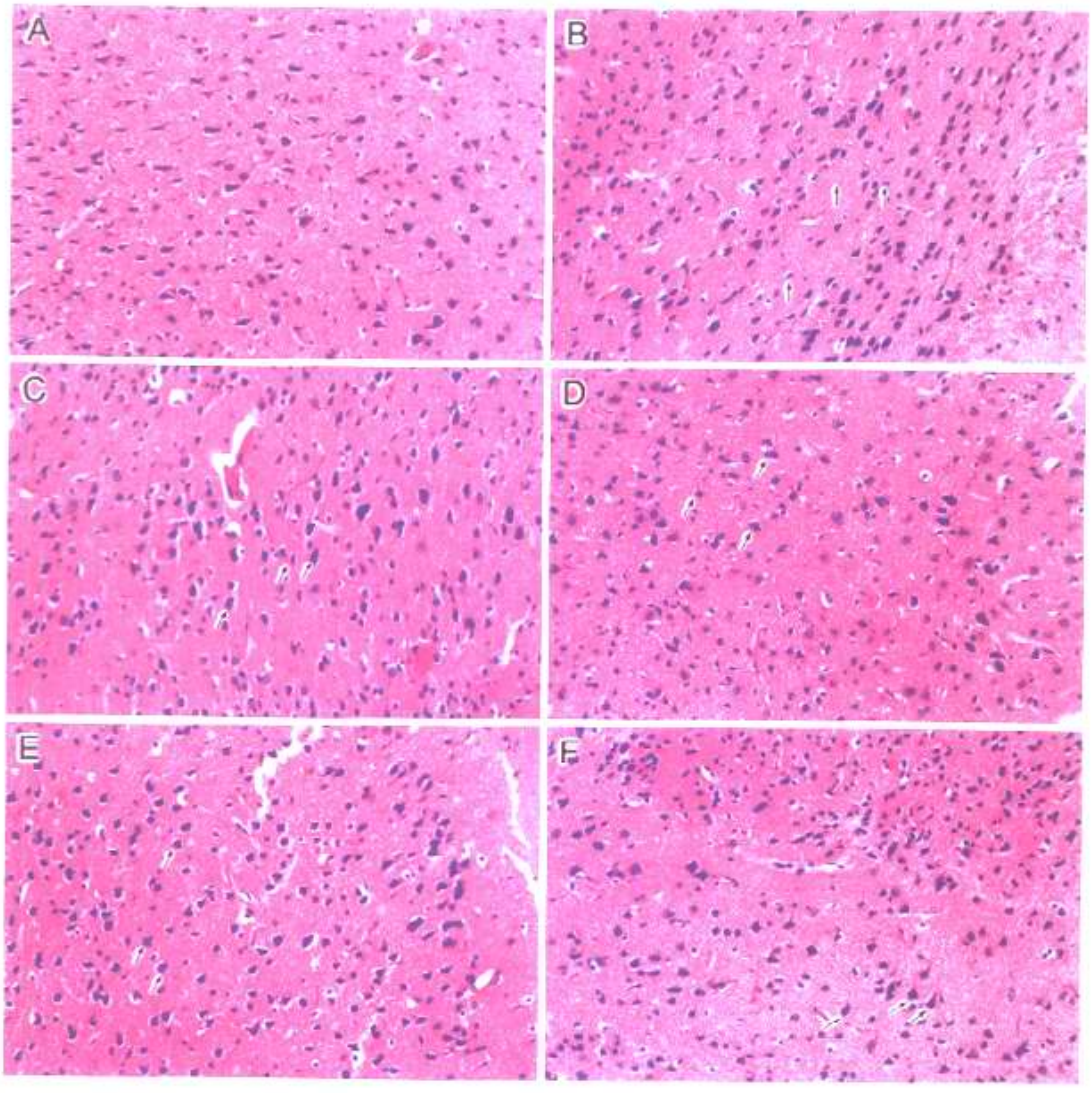

Fig 5. Representative photomicrographs of pyramidal cells in the cerebral cortex of mice after administration of galactose (120 mg/kg, ip) for 47 d. A: saline-treated group. B: galactose-treated group: the pyramidal cells became rounded, the cytoplasm was shrunken, the neurofibillary shortened, and the nuclei was side-moved and dark-stained (arrows: ↑). C: PIR-treated group; D, E, F: SSF-treated group at dose of 50, 100, and 200 mg/kg, respectively. PIR and three dose of SSF could all protect the cerebral cortex cells. The pyramidal cells became full, the neurofibillary lengthened, and the nuclei were light-stained and shaped variously (arrows: ↗). Especially, the protective effects in dose of SSF 200 mg/kg mice were much better than every other group. HE stain, × 200.

the cerebral cortex, which had been demonstrated to be correlated to the deficits in learning and memory. These results were also consistent with the notion that the neural system integrity was critical for intellectual function such as learning, memory, and cognition[9,12,13,19]. In our studies, long-term administration of SSF significantly improved water maze performance and attenuated histological lesions in the brain in mice caused by galactose. Its improving effect produced current in a dose-dependent manner against the neuron lesions, which were characteristics of most cognitive enhancers. The dose of SSF 200 mg/kg with the maximal cognitive improvement was paralleled with neuropathological attenuation. It seemed likely that the beneficial effects of SSF on cognitive deficits induced by galactose were due primarily to its improving effects on neuronal system.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Dec; 22 (12)
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There was evidence showing that immunodeficiencies were capable of mediating the brain senescence in humans such as Alzheimer's disease and vascular disease[10,20]. The immunodyfunction of animals was also observed in various dementias such as naturally occurring aging and ischemia and some chronic chemical regents induced (aluminium or galactose) dementia model[19,21-22]. In the present study, we found the spleen weight was significantly decreased in galactose mice. SSF (50, 100, and 200 mg/kg) could significantly raise the spleen weight in those mice. The results demonstrated that SSF could significantly improve the immune system and might contribute to attenuation of the impaired memory. These present findings, coupled with anti-hyperxia[15], suggested that SSF might have potential value in the treatment of senescence-related memory disorders such as Alzheimer's disease.

ACKNOWLEDGEMENTS To Assoc Prof CHEN Si-Ping for preparation of SSF and Prof ZHAO Shu-Min for observation of pathological slices.

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SSF 改善衰老小鼠记忆缺陷和神经、免疫系统的病理改变

商亚珍[1]，龚明玉，周晓霞，李素婷，王宝元
(河北承德医学院中药研究所，承德 067000，中国)

关键词 黄芩；记忆障碍；迷宫学习；半乳糖；神经免疫调节

目的：研究黄芩中有效成份 SSF 对衰老小鼠记忆障碍和神经、免疫系统病理改变的作用. **方法**：采用水迷宫行为实验法，检测 SSF 对衰老小鼠学习、记忆障碍的作用；大脑皮层光镜检测 SSF 对衰老小鼠神经病理改变的作用；胸腺和脾指数评价 SSF 对衰老小鼠免疫系统的作用；脑复康作对照药. **结果**：与盐水对照组相比，*D*-半乳糖(120 mg/kg，ip，47 d)使小鼠到达终点平台潜伏期和进入盲端的次数显著增加，大脑皮层细胞明显病理改变，脾指数减小. SSF (50，100 和 200 mg/kg，ig，47 d)和脑复康(200 mg/kg，ig，47 d)均可改善 *D*-半乳糖引起小鼠记忆障碍和神经、免疫系统异常改变. **结论**：SSF 能改善 *D*-半乳糖引起小鼠学习记忆障碍和神经、免疫系统的病理改变.

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