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Effects of puerarin on *D*-galactose-induced memory deficits in mice[1]

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KEY WORDS puerarin; galactose; memory; superoxide dismutase; malondialdehyde; lipofuscin

ABSTRACT

AIM: To study the effects of puerarin on learning-memory behavior in aging mice induced by *D*-galactose and to explore in-brain mechanism of its effects. **METHODS**: The aging mice model were induced by sc *D*-galactose 0.12 g/kg daily for 6 weeks and meanwhile treated with three doses of puerarin once a day for 6 weeks. The spontaneous behavior and the learning-memory behavior were tested for the aging mice using open field and Y-maze on the day after the last treatment. Then the activity of superoxide dismutase (SOD) and the contents of malondialdehyde (MDA) and lipofuscin in brain tissue were measured using UV-photospectrometer and fluorospectrophotometer analysis system. **RESULTS**: Compared with the *D*-galactose control group, puerarin 60 mg/kg was shown to increase significantly the spontaneous behavior and explorative response in the open field and improve remarkably the learning-memory ability of the aging mice induced by *D*-galactose. The percentage of memory retention increased from 69 % ± 9 % to 79 % ± 6 %. Puerarin 30 mg/kg and 60 mg/kg promoted remarkably the activity of SOD in the brain of the aging mice from (12.1 ± 2.9) to (14.9 ± 2.1) and (15.5 ± 2.7) $U \cdot g^{-1}$ wet brain weight, respectively, and decreased significantly the content of lipofuscin from (27 ± 5) to (20 ± 4) and (20 ± 4) $\mu g \cdot g^{-1}$ wet brain weight, respectively. **CONCLUSION**: Puerarin could improve the memory dysfunction produced by *D*-galactose. Improvement of the antioxidase activity of brain in the aging mice may be involved in this effect.

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INTRODUCTION

Cognitive deficits and tardiness in responses were the main clinical performances of brain senescence in human being. The memory dysfunction in senescent animals was found in various test of learning and memory[1-3]. There was evidence showing that galactose could result in senescent performances, retrograde changes in neural cell, and memory dysfunction in animals[4-7]. These produced senescent mechanisms might be correlated with lipid peroxidation[6,7]. Work was done in many countries to search for improvement of age-related memory dysfunction. Puerarin (Pue), 8-C-C-glucopyranosyl-4′-7-dihydroxyisoflavone, an active component extracted from the Chinese traditional medicine *Pueraria lobata* (wild) Ohwi, has been shown to be effective in treatment of cerebral ischemia[8], inhibition of lipid peroxidation by brain injury[9], and protection of neural cells and astrocytes against injury by Glu[10,11]. Pue could block transient outward K^+ current and delayed rectifier K^+ current in mice hippocampal CA_1 neurons[12]. However, little is known about the influence of Pue on memory dysfunction. The present study was to investigate the effect of Pue on memory deficits and probe into its possible mechanism of improving brain function.

MATERIALS AND METHODS

Subjects and chemicals Kunming mice of both sexes, weighing (25.0 ± 2.0) g, were purchased from Experimental Animal Center, Zhejiang Academy of Medical Science (Grade Ⅱ, Certificate № 99002). *D*-galactose (*D*-gala) was from the Second Chemical Works in Shanghai. Puerarin injection (Pur, lot № 99091) was from Conba Pharmaceutical Co. All other chemicals were of AR grade.

Drug administration Having adapted the conditions in our laboratory, 120 mice were randomly divided into five groups, 24 animals in each group.

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Animals with sc and ip saline (0.3 mL each mouse) served as normal control group (Group NC); animals with sc *D*-gala (0.12 g/kg) and ip saline (0.3 mL each mouse) served as aging control group (Group AC); animals with sc *D*-gala (0.12 g/kg) and ip Pue (15 mg/kg, 30 mg/kg, 60 mg/kg) served as aging Pue treatment groups (Group PT15, Group PT30, Group PT60)/ The aging mice were induced by sc *D*-gala daily for 6 weeks[7], meanwhile, the treatment with Pue also last for 6 weeks.

Open field behavior On the day next to the last treatment with Pue, spontaneous behavior of mice were tested using open field[13]. The open field was divided into 13 checks. The mouse was placed into the central check, then the check of locomotion was recorded that the mouse moved through in the open field in 1 min and 3 min, the frequencies of rearing and grooming, and the pellets of defecation in 3 min were recorded too, respectively.

Y-maze training Y-maze task was used to examine the learning-memory behavior of all animals[13]. It is regarded as "correct response" that the mouse directly moved into the secure arm of Y-maze when it suffered from footshock (0.4 mA). The mice were trained till they had reached the learning criterion, namely there were 9 times "correct response" in 10 times training continuously. The less the times of training need, the stronger capability of learning is. The memory retention was tested 24 h after training.

Measurement of SOD activity and the MDA level in brain Having examined the memory behavior, mice were decapitated, and their brains were removed and homogenized in saline. The homogenate (20 %) was centrifuged at 4500 × *g* at 4 ℃ for 20 min, and the supernatant was used for superoxide dismutase (SOD) and malondialdehyde (MDA) assay by UV-photospectrometer (the Analytic Instrument Works in Shanghai). The assay of SOD activity was based on its ability to inhibit the oxidation of pyrogallicacid[14]. One unit of SOD activity was defined as the amount that reduced the optical density (*OD*) at 325 nm by 50 % and results were expressed as units per gram of wet brain. The level of lipid peroxidation in brain homogenate was indicated by the content of MDA in brain tissue. Thiobarbituric acid reaction (TBAR) method was used to determine the MDA (at 532 nm)[15]. MDA content was expressed as nmol per gram of wet brain.

Measurement of the level of brain lipofuscin The mice brains were homogenized in 7 mL of 2:1 mixture of chloroform-methanol. The resulting brei was centrifuged at 1500 × *g* for 20 min, the top layer was discarded and the remaining was adjusted to 7 mL by adding chloroform-methanol mixture. Spectrofluorometric measurements were made with RF-540 spectrofluorometer (Shimadzu Co, Japan) at an excitation maximum of 365 nm and an emission maximum of 435 nm[16]. The fluorescence intensity of the extracts was calculated in relation to 1 g wet brain per liter of chloroform-methanol mixture in the original extraction. The results were expressed as μg lipofuscin per g of wet brain. The spectrofluorometer was standardized each time with a fresh solution of quining bisulphate (1 mg/L).

Statistical analysis The results were expressed as $\bar{x} \pm s$ and analyzed by unpaired *t*-test.

RESULTS

Effect of Pue on open field behavior Compared with Group NC, the check of locomotion that the aging mice moved in 1 min and 3 min, and the frequency of rearing were significantly decreased. After treatment with Pue 60 mg/kg, the check of locomotion, the frequency of rearing, and the pellet of defecation of aging mice were all drastically increased (Tab 1).

Tab 1. Effects of Pue (15, 30, 60 mg/kg, ip, for 6 weeks) on open field behavior of the aging mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **Group AC.**

Group	*n*	Locomotion (check) 1 min	Locomotion (check) 3 min	Rearing (frequency)	Grooming (frequency)	Defecation (pellet)
Group NC	9	29 ± 11^b	70 ± 34^b	16 ± 8^c	2.9 ± 1.8	3.7 ± 1.8
Group AC	10	15 ± 7	39 ± 18	6 ± 4	1.1 ± 1.5	2.3 ± 2.2
Group PT 15	12	18 ± 8	57 ± 22	9 ± 4	1.8 ± 1.3	4.8 ± 2.7^b
Group PT 30	10	20 ± 11	52 ± 20	12 ± 6	1.4 ± 1.1	4.6 ± 1.1^b
Group PT 60	12	22 ± 9^b	65 ± 25^b	14 ± 7^c	1.6 ± 1.8	4.5 ± 2.0^b

Effect of Pue on learning-memory behavior

The training frequency reached the learning criterion of the aging mice was remarkably increased compared with Group NC and the percentage of memory retention was declined. After treatment with Pue 60 mg/kg, the training frequency was significantly reduced and the percentage of memory retention was promoted (Tab 2).

Tab 2. Effects of Pue (15, 30, 60 mg/kg, ip, for 6 weeks) on the learning-memory behavior of the aging mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* Group AC.

Group	*n*	Training times of reaching standard (frequency)	Percentage of memory retention/%
Group NC	11	26 ± 9^b	78 ± 9^b
Group AC	12	36 ± 10	69 ± 9
Group PT 15	10	32 ± 12	71 ± 8
Group PT 30	10	27 ± 12	78 ± 10^b
Group PT 60	10	26 ± 8^b	79 ± 6^c

Effects of Pue on the activity of SOD and the contents of MDA and lipofuscin in brain tissue

Pue 30 and 60 mg/kg remarkably improved the activity of SOD and reduced the level of lipofuscin in the aging mice brain, but had no significant effect on the level of MDA in brain (Tab 3).

DISCUSSION

The present study showed that long-term administration of *D*-galactose induced amnesia and tardiness in responses in mice, and their spontaneous behavior and explorative response in the open field decreased significantly, and their ability of learning-memory declined remarkably, too. We also found that chronic galactose induced decrease of SOD activity and increase of MDA and lipofuscin level in brain. These results were consistent with the impaired memory in the aging mice induced by *D*-galactose. Many papers have reported that lipid peroxidation in brain tissue may contributed to galactose-induced memory impairments[6,7]. The metabolite of *D*-galactose can not be further metabolized and accumulate in nervous cells, and a great deal of free radicals resulted from oxidation of *D*-galactose surpass cells' ability of cleaning them. This, consequently, caused the chain reaction of lipid peroxidation (LPO) and the product that resulted from dialysis of LPO, such as MDA, could combine with protein and phospholipid, so lead to injury of cellular membrane and the function impairment of neural system[17]. In the present study, long-term administration of Pue significantly improved Y-maze performance and increased the spontaneous behavior in aging mice caused by *D*-galactose, and promotion of SOD activity and decline of lipofuscin level were paralleled with improvement of memory dysfunction.

The results indicated that Pue had protective effect against memory dysfunction induced by *D*-galactose through raising the SOD activity in brain, promoting the ability of cleaning free radicals, inhibiting the chain reaction of lipid peroxidation, and decreasing accumulation of lipofuscin in brain of aging mice. The study suggested that Pue might have potential value in the treatment of senescence-related memory disorders such as Alzheimer's disease.

Tab 3. Effects of Pue (15, 30, 60 mg/kg, ip, for 6 weeks) on the activity of SOD and the contents of MDA and lipofuscin in brain tissue of aging mice. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* Group AC.

Group	*n*	SOD/U·g^{-1} wet weight	MDA/nmol·g^{-1} wet weight	Lipofuscin/μg·g^{-1} wet weight
Group NC	11	16.3 ± 2.3^c	1.6 ± 0.3^b	22 ± 4^b
Group AC	12	12.1 ± 2.9	1.81 ± 0.14	27 ± 5
Group PT 15	10	13.2 ± 2.4	1.8 ± 0.3	23 ± 6
Group PT 30	10	14.9 ± 2.1^b	1.68 ± 0.28	20 ± 4^c
Group PT 60	10	15.5 ± 2.7^b	1.08 ± 0.28	20 ± 4^c

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葛根素 *D*-半乳糖诱导的小鼠记忆障碍的影响[1]

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关键词 葛根素；半乳糖；记忆；超氧化物歧化酶；丙二醛；脂褐素

目的：探讨葛根素对 *D*-半乳糖致衰老小鼠学习记忆的影响及其作用机制。**方法**：用开场行为和 Y-迷宫分别检测腹腔注射葛根素后 *D*-半乳糖衰老模型小鼠自发活动和学习记忆行为的变化，然后用紫外分光光度计和荧光分光光度计分别检测衰老小鼠脑组织 SOD、MDA 以及脂褐素等生化指标。**结果**：葛根素 60 mg/kg 可显著增加 *D*-半乳糖致衰老小鼠在新异环境中的自发活动和探究行为，显著提高其学习记忆能力（记忆保持率从 69 % ± 9 % 提高到 79 % ± 6 %）；葛根素 60 mg/kg 和 30 mg/kg 均可以显著提高脑组织 SOD 的活性，从 (12.1 ± 2.9) 提高到 (14.9 ± 2.1) 和 (15.5 ± 2.7) U/g 脑湿重，并使衰老小鼠脑内脂褐素含量明显下降，从 (27 ± 5) 降到 (20 ± 4) 和 (20 ± 4) μg/g 脑湿重。**结论**：葛根素对 *D*-半乳糖致衰老小鼠学习记忆减退有改善作用，这可能与其提高衰老小鼠脑组织抗氧化能力有关。

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