Effects of *Panax ginseng* and Its Constituents on Drug-induced Memory Impairment in Rats

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ABSTRACT

In this present study, we investigated the effects of red ginseng extract and its active constituents - Rb_1, Re, Rg_1 on cycloheximide (CXM)-induced amnesia in the passive avoidance task in rats.

Red ginseng water extract at 0.05-0.5 g/kg could improve CXM-induced amnesia in rats. Furthermore, the recovery effect of Rb_1 at 10 mg/kg administered 30 min before training trial from CXM-induced amnesia was better than those of Rb_1 administered other time before or after training trial. Rb_1 at 0.001-0.1 mg/kg could significantly improve CXM-induced amnesia and at 1 mg/kg completely augmented, but at 10 mg/kg its improving effect slightly weakened. Rg_1 and Re at 0.3-10 mg/kg could significantly improve CXM-induced amnesia and Rg_1 at 10 mg/kg completely augmented. On the other hand, Rb_1 at 10 mg/kg could prolong the step through latencies in the training trial.

These results suggest the beneficial effect of red ginseng extract on CXM-induced amnesia in rats could mainly due to the contribution of its active constituents - Rb_1, Re, Rg_1. The improving effect of Rb_1 on CXM-induced amnesia was best among the three active constituents. But the reduction in the improving effect of Rb_1 at 10 mg/kg might be due to the decrease in motor activity and attention to the passive avoidance task.

Introduction

Ginseng Radix, the root of *Panax ginseng* C.A. Meyer, was originally described in the Shen-Nung-Pen-Ts'ao-Ching in ancient China. It was used to build up strength and remove fatigue in traditional medicine. Modern pharmacological studies suggested that Ginseng Radix has bi-directional regulation effects in CNS, anti-fatigue, anti-hypertensive and decreases peripheral blood resistance effect (Cheng, 1989). Furthermore, recent study suggested that ginseng improved the drug-induced deficit after three-day consecutive administration on passive avoidance performance in rats (Ma, 1990). In clinical reports, it enhances cognition and improves memory dysfunction in dementia (Yang, 1994). From recent studies for cognitive activators, it became clear that neurotrophic factors,

enhancing neuronal differentiation, plasticity and repair, and ganglioside improved passive avoidance retention after chronic administration, but not after acute administration.

Memory processes are usually divided into three stages, such as acquisition, consolidation and retrieval. Alteration of neurotransmitters such as acetylcholine, dopamine, serotonin and synthesis of brain new proteins have distinct levels and orientation in the three memory stages (Izquierdo, 1984; Jodar and Kaneto, 1995). In learning acquisition process, the cholinergic neuronal system plays an important role in humans and animals (Drachman and Leavitt, 1974; Bartus *et al.*, 1982; Fibiger, 1991). Scopolamine (SCOP) is a muscarinic antagonist, decreases cholinergic activity and impairs learning and memory in rodents and humans, especially in learning acquisition (Elord and Buccafusco, 1988; Okaichi *et al.*, 1989; Rush, 1988). Therefore, SCOP is used as a drug to induce the impairment of on learning acquisition. For memory consolidation process, brain protein synthesis is an essential step followed by various forms of training (Dunn, 1980). Cycloheximide (CXM) is a protein synthesis inhibitor, impairs memory consolidation and long-term memory in rodents (Chapouthier, 1983; Davis and Squire, 1984). Therefore, CXM is used as a drug to induce the impairment of memory consolidation. In memory retrieval process, the dopaminergic system plays an important role and the decrease in dopaminergic activity facilitates memory retrieval (White *et al.*, 1993). Apomorphine (APO) is a dopamine receptor agonist in high dose, increases dopaminergic activity and impairs memory retrieval in rodents (Ichihara *et al.*, 1988). Therefore, APO is used as a drug to induce the impairment of memory retrieval. In the present study, we investigated 1) the effects of red ginseng extract after acute administration on learning and memory process such as acquisition, consolidation and retrieval in passive avoidance task impaired by SCOP or CXM or APO, and used piracetam as a positive control. 2) the effects of ginsenosides (Rb_1, Re and Rg_1) on CXM-induced memory consolidation impairment.

Materials and Methods

1. Plant preparation

Red Ginseng Radix (3 kg) were chopped and extracted with distilled water plus a little 95% alcohol (35 l) by maceration (2 weeks) and the extract reduced to dryness with a vacuum rotary evaporator. A yield of 1,048.8 g (34.96%) was obtained.

2. Animals

Male Sprague-Dawley rats, weighing 200-250 g, were housed in groups of six with free access to food and water and kept in a regulated environment (23 ± 1℃), wherein a 12 hr light-dark cycle (8:00 to 20:00, light) was maintained.

3. Apparatus

Rats were trained in a step-through passive avoidance task. The apparatus consisted of two compartments having a steel-rod grid floor (36 parallel steel rods, 0.3 cm in diameter set 1.5 cm apart). One of the compartments (4820 30 cm) was equipped with a 20W lamp located centrally at a height of 30 cm, and the other was dark compartment of same size, connected through a guillotine door (55 cm). The dark room was used during the experimental sessions that were conducted between 09:00 and 17:00 hours.

4. Passive avoidance performance

During the trial training, the guillotine door connecting the light and dark compartment was kept 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 to enter the dark compartment was measured with a stopwatch. Once the rat entered the dark compartment, the door was closed. An inescapable scrambled footshock (1.0 mA for 2 s) was then delivered through the grid floor. The rat was removed from the dark compartment 5 s after administering the shock. The rat was then put back into its home cage until the retention trial, which was carried out twenty-four hours later. The rat was once again placed in the light compartment and as in the case of training trial, the guillotine door was opened and the step-through latency was recorded and used as a measure of retention. An upper cut-off time of 300 sec was set.

In the first experiment, SCOP (1 mg/kg, i.p.) was administered 30 minutes before the training trial (Elrod and Buccafusco, 1988). In the second experiment, CXM (1.5 mg/kg, s.c.) was administered immediately after the training trial (Davis and Squire, 1984). In the third experiment, APO (1 mg/kg, i.p.) was administered 20 minutes before the retention trial (Ichihara *et al.*, 1988). Red ginseng extract (0.015 - 0.5 g/kg, i.p.) was administered to the rats 30 min before the training trial. Piracetam (50, 100 and 300 mg/kg, p.o.) was also administered to the rats 1 hr before the training trial.

Rb_1 (10 mg/kg, i.p.) was administered to the rats 30 min and 1 hr before the training trial or immediately and 15 min after the training trial. Then, ginsenosides (Rb_1, Re and Rg_1; 0.001 - 10 mg/kg, i.p.) was administered to the rats 30 min before the training trial.

5. Non-shocked rats

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 rats were not subjected to 1.0 mA inescapable footshock during the training period. Twenty-four hours later, the retention trial was carried out and the step-through latency was recorded (Ichihara *et al.*, 1988). The rats were given various doses of red ginseng extract and ginsenosides in combination with CXM.

6. Chemicals

Rb_1, Re and Rg_1 were kindly provided by Korea Ginseng & Tobacco Research Institute, Piracetam (PIR; Sigma), scopolamine hydrobromide (SCOP; Sigma), cycloheximide (CXM; Sigma) and *p*-chloroamphetamine hydrochloride (PCA; Sigma) were all dissolved in 0.9% saline.

7. Statistics

All data obtained during the passive avoidance task was expressed in terms of medians and interquartile ranges and further analyzed by using a Kruskal-Wallis non-parametric one-way analysis of variance, followed by Mann-Whitney' s U-test. In addition, the data collected during motor activity and shock sensitivity was analyzed using a one-way analysis of variance, followed by Scheffe multiple range test. The criterion for statistical significance was $P < 0.05$ in all the above evaluations.

Result

1. Effect of red ginseng extract on drug-induced memory processes impairment in rats

It was observed that pretraining administration of SCOP (1 mg/kg, i.p.) remarkably reduced the step-through latencies in the retention test. The effects of pretraining administration of red ginseng extract and piracetam on retention latencies which were shortened by SCOP are shown in Fig. 1. The shortened latencies induced by CXM were reversed by pretraining administration of red ginseng extract at 0.05-0.5 g/kg, The plot of dose-response relationship for this effect of red ginseng extract was a bell-shaped curve, with a maximal effect occurring at 0.05 g/kg. Pretraining administration of at 100 mg/kg also significantly attenuated the SCOP-induced impairment of memory.

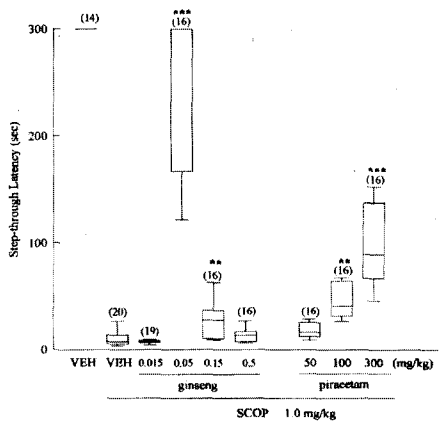

Fig. 1. Effects of red ginseng extract and piracetam on the SCOP-induced learning acquisition impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. * P<0.05, *** P<0.001 compared with SCOP group.

It was observed that administration of CXM (1.5 mg/kg, s.c.) remarkably reduced the step-through latencies in the retention test. As shown in Fig. 2, the shortened latencies induced by CXM were reversed by pretraining administration of red ginseng extract at 0.05-0.5 g/kg. Pretraining administration of piracetam at 100 mg/kg significantly attenuated the CXM-induced impairment of memory.

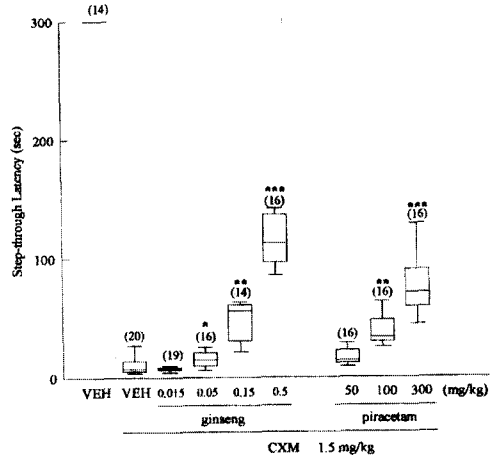

Fig. 2. Effects of red ginseng extract on the CXM-induced memory consolidation impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *** P<0.001 compared with VEH/CXM group.

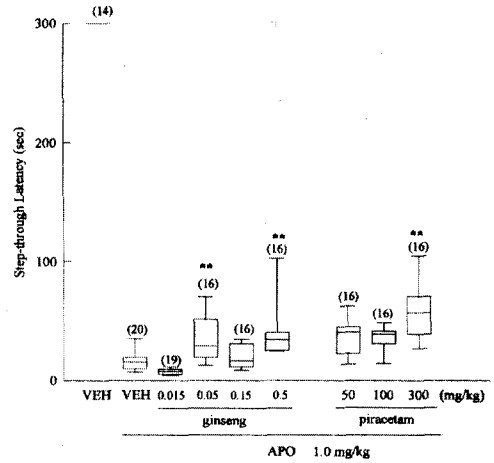

Fig. 3. Effects of red ginseng extract and piracetam on the APO-induced memory retrieval impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. * P<0.05, ** P<0.01, *** P<0.001 compared with APO group.

cantly attenuated the CXM-induced impairment of memory.

It was observed that pretesting administration of APO (1 mg/kg, i.p.) remarkably reduced the step-through latencies in the retention test. As shown in Fig. 3, the shortened latencies induced by APO were reversed by pretesting administration of red ginseng extract at 0.05-0.5 g/kg. Pretraining administration of piracetam at 300 mg/kg significantly attenuated the APO-induced impairment of memory.

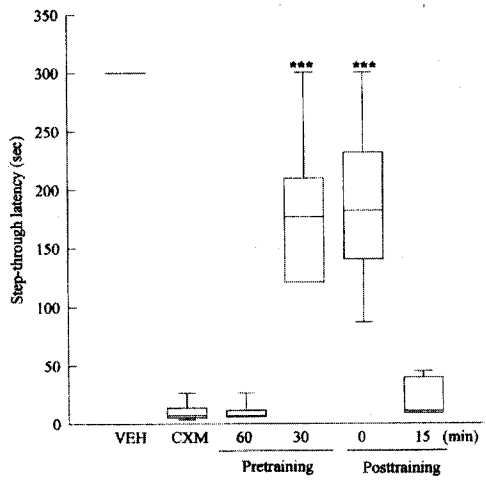

Fig. 4. Effects of Rb_1 on the CXM-induced memory consolidation impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *** P<0.001 compared with CXM group.

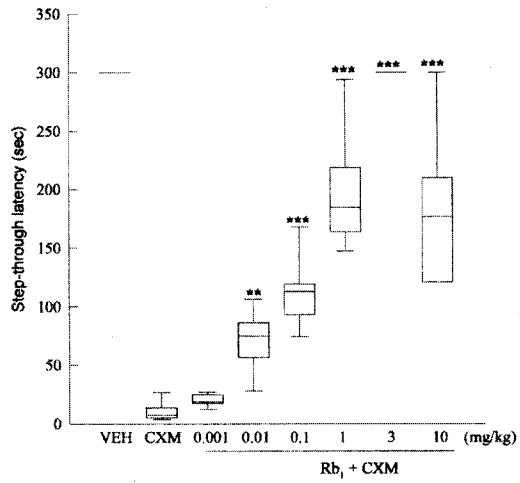

Fig. 5. Effects of Rb_1 (0.001-10 mg/kg) on the CXM-induced memory consolidation impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *** P<0.001 compared with CXM group.

2. Effect of ginsenosides on CXM-induced memory processes impairment in rats

As shown in Fig. 4, the shortened latencies induced by CXM were reversed by Rb_1 at 10 mg/kg 30 min before the training trial or immediately after the training trial, but not 60 min before the training trial and 15 min after the training trial.

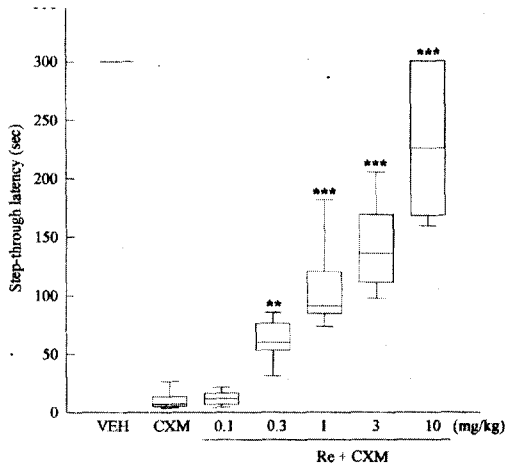

Fig. 6. Effects of Re (0.1-10 mg/kg) on the CXM-induced memory consolidation impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *** P<0.001 compared with CXM group.

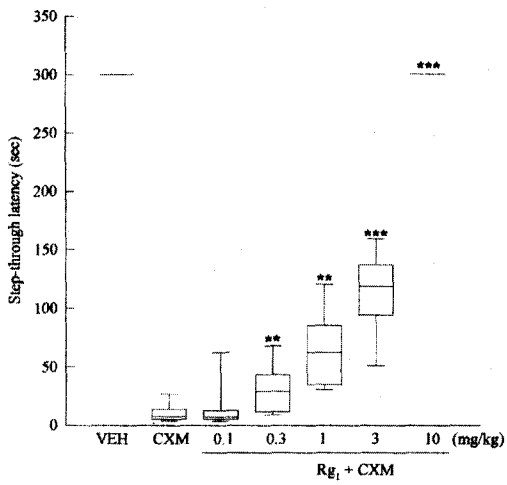

Fig. 7. Effects of Rg_1 (0.1-10 mg/kg) on the CXM-induced memory consolidation impairment of inhibitory avoidance response in rats. Each column represents the medians and the range inside 5th and 95th percentile. *** P<0.001 compared with VEH group.

As shown in Fig. 5, the shortened latencies induced by CXM were reversed by pretraining administration of Rb_1 at 0.001-1 mg/kg, at 1 mg/kg could completely augment CXM-induced shortened latencies. But the effect of Rb_1 at 10 mg/kg slightly reduced. As shown in Fig. 6, the shortened latencies induced by CXM were reversed by pretraining administration of Re at 0.1-10 mg/kg. As shown in Fig. 7, the shortened latencies induced by CXM were reversed by pretraining administration of

Table 1. Effects of red ginseng extract and ginsenosides (Rb_1, Re and Rg_1) on the step-through latency of non-shocked rats given CXM.

Drug	Dose (mg/kg, IP)	Step-through Latency (sec) Training trial
Control		10.9560.34
CXM	1.5	12.191.26
Red ginseng extract	50	7.770.62
+ CXM	150	7.232.09
	500	13.893.32
Rb_1 + CXM	0.001	11.371.32
	0.01	17.611.74
	0.1	14.352.45
	1	12.941.44
	3	16.292.27
	10	34.151.98*
Re + CXM	0.1	18.132.64
	0.3	11.081.88
	1	13.392.55
	3	15.693.35
	10	18.593.65
Rg_1 CXM	0.1	9.531.76
	0.3	13.162.41
	1	11.451.23
	3	17.283.73
	10	19.196.31

The result expressed as mean S. E. N=8, *P<0.05 compared with CXM group.

Rg_1 at 0.1-1 mg/kg, at 10 mg/kg could completely augment CXM-induced shortened latencies.

3. Non-shocked rats

Pretraining administration of red ginseng extract and ginsenosides (Rb_1, Re, and Rg_1) both had no effect on the step-through latencies of non-shocked rats which were given CXM, except that Rb_1 at 10 mg/kg prolonged the step-through latencies in the training trial (Table 1).

Discussion

Learning and memory processes such as acquisition, consolidation and retrieval are related to modification of neurotransmitters such as acetylcholine, dopamine, serotonin and synthesis of new protein (Izquierdo, 1984; Jodar and Kaneto, 1995). It has long been suggested that the cholinergic neuronal system plays a major role in learning and memory in humans and animals, especially in learning acquisition (Fibiger, 1991; Elrod and Buccafusco, 1988). In the present study, it was observed that pretraining administration of SCOP significantly shortened the step-through latency of the retention trial in passive avoidance task. Pretraining administration of red ginseng extract prolonged the step-through latency shortened by SCOP. Pretraining administration of piracetam at 100 mg/kg, a nootropic drug, prolonged the step-through latency shortened by SCOP. The effect of piracetam is consistent with the results of earlier study (Piercey *et al.*, 1987) and partially due to the increase of acetylcholine release (Gouliaev and Senning, 1994).

Secondly, various forms of training, memory consolidation and long-term memory are followed by an increased rate of brain protein synthesis (Dunn, 1980; David and Squire, 1984). In the present study, it was observed that CXM immediately after training significantly shortened the step-through latency of the retention trial in the passive avoidance task. Red ginseng extract prolonged the step-through latency shortened by CXM in passive avoidance task. The effect of red ginseng extract after acute administration is consistent with the results of earlier study after three-day consecutive administration. Additionally, the dopaminergic system plays an important role in memory processes. The decrease in dopaminergic activity facilitates memory retrieval (Ichihara *et al.*, 1988). In the present study, it was observed that APO administrated 20 min before the retention trial significantly shortened the step-through latency of the retention trial in passive avoidance task. Red ginseng extract prolonged the step-through latency which was shortened by APO in passive avoidance task. The present data demonstrated that red ginseng extract could improved the SCOP-induced impairment of learning acquisition, the CXM-induced impairment of memory consolidation and the APO-induced impairment of memory retrieval in passive avoidance task.

On the other hand, several investigators have suggested that the amnesia action of CXM might be due to disturbance of the catecholaminergic neuronal system, cholinergic neuronal system and the increase of the serotonergic activity in experimental animals. The effects of CXM on catecholaminergic synthesis were due to the selective inhibition of norepinephrine synthesis. Therefore, the improving effects of red ginseng extract after acute administration on learning and memory processes might be related to the decrease in dopamine concentration or other monoamine concentration, the increase in acetylcholine concentration and prevent DNA degradation. Therefore, the detailed action mechanism of the facilitating effects of red ginseng extract on memory processes will be further investigated.

Furthermore, Rb_1 (10 mg/kg) 30 min before the training trial or immediately after the training trial could reverse the shortened latencies induced by CXM, but not 60 min before the training trial and 15 min after the training trial. Therefore, Rb_1 could mainly contribute to memory consolidation but not learning acquisition. Rb_1 at 0.001-0.1 mg/kg could significantly reverse the CXM-induced shortened latencies, and at 1 mg/kg completely augmented the CXM-induced shortened latencies. But the effect of Rb_1 at 10 mg/kg slightly reduced. Secondly, Re at 0.1-10 mg/kg could significantly reverse the CXM-induced shortened latencies. Finally, Rg_1 at 0.1-1 mg/kg could significantly reverse the CXM-induced shortened latencies, and at 10 mg/kg completely augmented the CXM-induced shortened latencies.

On the other hand, the effect on motivational or motor systems can in turn affect the acquisition of the avoidance response. Several aspects argue against the possibility that the passive avoidance response in drug-treated animals can be related with motor alteration during training. The behavior observed in the passive avoidance task which received red ginseng extract or ginsenosides (Rb_1, Re and Rg_1) in combination with CXM was grossly normal in the training or retention trial, but Rb_1 at 10 mg/kg in combination with CXM prolonged the step-through latency in the training trial. Therefore, from the present results it suggested that ginseng or Re and Rg_1 improving effects in CXM-induced impairment mainly were related to memory-related process, but Rb1 in high dose (10 mg/kg) might selectively alter the motor activity and attention in passive avoidance task.

In summary, red ginseng extract after acute administration facilitated learning acquisition, memory consolidation and retrieval in passive avoidance task. Rb_1, Re and Rg_1 are the active constituents of red ginseng extract on learning and memory processes. Rb_1 is the better constituent than other two constituents and mainly affect the memory consolidation process.

Acknowledgement

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