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Effect of Korean Red Ginseng and Crude Drug-Combined Preparations (RGCDPs) on Memory Enhancement in Mice

Jae Joon Wee, Jong Soo Kyung, Na Mi Kim, Yong-Bum Song, Yi-Seong Kwak, and Jong Dae Park*

KT&G Central Research Institute, 302 Shinseong-dong, Yuseong-ku, Daejeon 305-805, Korea

Abstract – Anti-amnestic activities of Korean red ginseng (Ginseng Radix Rubra) and crude drug-combined preparations (RGCDP-1, RGCDP-2, and RGCDP-3) were evaluated by the animal experiment. RGCDP-1 and RGCDP-2 were prepared based on Korean folk prescriptions, "Chongmyongtang" and "Guibitang", respectively, while RGCDP-3, by a combination of both. Among the three preparations, RGCDP-3 was found to show the most potent anti-amnestic activity as evaluated by the passive avoidance test with mice, indicating synergistic action by combined effects of RGCDP-1 and RGCDP-2.

Keywords – Korean red ginseng, *Panax ginseng*, crude drug preparation, memory enhancement, passive avoidance test, synergistic effect

Introduction

Panax ginseng, regarded as a tonic with antistress, antifatigue and antiaging properties, has been intensively studied on its nootropic activity. A number of studies suggest that ginseng can be effective in the attenuation of learning deficits due to brain damage and ageing in rodents (Zhao *et al.*, 1998; Zhong *et al.*, 2000). In clinical study, improved mnemonic performance (Randt Memory Test) was observed in the cohort suffering from age-related memory impairment (Neri *et al.*, 1995).

Other medicinal plants used for cognitive benefits are Polygalae Radix, Acori Graminei Rhizoma, Zizyphi Spinosi Semen, Hoelen, which are main ingredients of "Chongmyongtang", one of prescriptions in "Dongeui bogam". Alcohol extract of Polygalae Radix was reported to be effective in the attenuation of learning deficits due to brain damage (Park *et al.*, 2002). Acori Graminei Rhizoma extract and its essential oil was reported to protect PC-12 cells from the toxic effect of amyloid-β, peptide, implying the beneficial potentials for the management of Alzheimer disease (Irie *et al.*, 2003).

On the other hand, Korean folk prescriptions, such as "Guibitang" and "Bojungikgitang", have been used for enhancement of the deficiency of vital energy. They include such ingredients as Ginseng Radix, Astragalus Radix, Angelicae Gigantis Radix, Atractylodes Rhizoma Alba, Zizyphi Fructus, Glycyrrhizae Radix in common. These findings as well as the above-mentioned reports have led us to prepare a Korean red ginseng and crude drug-combined preparation (RGCDP-3) by combining two crude drug prescriptions, "Chongmyongtang"-based RGCDP-1 and "Guibitang"-based RGCDP-2 that have been used for either brain function or vital energy enhancement, respectively.

The present study was undertaken to examine the synergistic effect of the combination of crude drug preparations (RGCDP-1 and RGCDP-2) on scopolamine-induced memory dysfunction in mice.

Experimental

Crude drugs – All crude drugs were purchased in a local market for Oriental medicines in Keumsan, Chungnam, Korea in 2002. The plant materials were authenticated by Dr. Ki Whan Bae, Professor, College of Pharmacy, Chungnam National University. Voucher specimens have been deposited in the Herbarium of Division of Ginseng Research, KT&G Central Research Institute.

Preparation of Korean red ginseng and crude drug-combined preparations (RGCDPs) – The ingredients of crude drug preparations RGCDP-1, RGCDP-2, and RGCDP-3 were listed in Table 1. Fifty gram of each crude drug preparation was extracted with 500 ml of water at 85°C for 6 h three times. The combined decoction was evaporated under reduced pressure and freeze-dried

*Author for correspondence
Fax: +82-42-861-1949; E-mail: jdpark@ktng.com

Table 1. The ingredients of crude drug preparations RGCDP-1, RGCDP-2, and RGCDP-3 and their composition

Ingredient	Composition (%)		
	RGCDP-1	RGCDP-2	RGCDP-3
Ginseng Radix Rubra (*Panax ginseng* C.A. Meyer)	27.2	24	9.1
Polygalae Radix (*Polygala tennuifolia* Willdenow)	18.2	-	5.5
Acori Graminei Rhizoma (*Acorus gramineus* Solander)	18.2	-	5.5
Zizyphi Spinosi Semen (*Zizyphus vulgaris*)	18.2	-	5.5
Hoelen (*Poria cocos* Wolf)	18.2	-	5.5
Schizandrae Fructus (*Schizandra chinensis* Baillon)	-	12	10.9
Astragalus Radix (*Astragalus membranaceus* Bunge)	-	16	14.5
Angelicae Gigantis Radix (*Angelica gigas* Nakai)	-	16	14.5
Atractylodes Rhizoma Alba (*Atractylodes japonica* Koidzumi)	-	16	14.5
Zizyphi Fructus (*Zizyphus jujuba*)	-	14	12.7
Glycyrrhizae Radix (*Glycyrrhiza uralensis* F.)	-	2	1.8

to obtain powdered extract. The yields of the extracts were 24, 30, 28%, respectively. The extract was then dissolved in distilled water (250 mg/ml) immediately before oral administration.

Animals and drug treatments –Male ICR mice (SAMTACO Experimental Animal Breeding Company, Suwon, Korea), weighing 25-30 g (6 weeks old), were used for passive avoidance tests following one-week adaptation period. Five or six male mice per cage were housed with free access to food and water and the mice were kept in a constant environment (22 ± 2°C, 50 ± 5% humidity, 12 h light cycle starting at 7 : 00 am). The scopolamine was purchased from Sigma (St. Louis, MO, USA) and were dissolved in sterile distilled water at a concentration of 500 ug/mL. Freeze-dried extract powders of RGCDPs were injected intraperitoneally to mice at doses of 60, 120, and 240 mg/kg body weight.

Passive avoidance test –The training apparatus consists of a 25×25×30 cm Plexiglas box with a 0.5 cm-high, 8 cm wide and 10 cm long platform on the left end of a series of bronze bars that make the floor of the box. During training, animals were gently placed on the platform facing the left rear corner of the training box. When they stepped down and placed their four paws on the grid, they received a 2 s, 0.5 mA scrambled footshock and were immediately retired to their home cages.

Fifteen mice were used per treatment. Sixty min before the training trial, mice received test samples by intraperitoneal injection. After 30 min, amnesia was induced in mice with scopolamine (2 mg/kg body weight, dissolved in 0.1% DMSO) given subcutaneously. Twenty-four hours after the training trial, the mice were again placed on the platform. The latency time to step down was measured. If the mice did not step down within 120 s, we concluded that the mice had memorized the passive avoidance training after one training trial (Christensen *et al.*, 1992; Kim *et al.*, 1999).

Data analysis – All data were expressed as mean ± SD. These data were analyzed by one-way ANOVA. In every case, the acceptable level for statistical significance was $p < 0.05$.

Results

Passive avoidance test – The step-down latency of the scopolamine-treated group was significantly shorter than that of the saline-treated control group (Tables 2, 3 and 4). The shorter step-down latency induced by scopolamine was significantly reversed by the treatment of RGCDP-1 at the dose of 240 mg/kg (Table 2). The latency time of RGCDP-2-treated mice, however, remained unchanged at any doses compared with non-treated group (Table 3). In contrast, RGCDP-3 was effective at all doses of 60, 120, 240 mg/kg, indicating that RGCDP-3 is more efficacious than RGCDP-1 against scopolamine-induced amnesia (Tables 2 and 4).

Discussion

Animal and human studies indicate that learning and memory can be modified by drugs affecting central cholinergic function (Bartus *et al.*, 1982; Kopelman & Corn, 1988; Fibiger, 1991). Scopolamine interferes with memory and cognitive function in humans and experimental animals by blocking muscarinic receptors (Beatty *et al.*, 1986; Wu *et al.*, 1996). In this experiment, we used

Table 2. Anti-amnestic effect of Korean red ginseng and crude drug-combined prescription, RGCDP-1, on scopolamine-induced memory impairment in mice

Experimental treatment[a]	Step down latency(s) (% of control)
Control[b]	73.2 ± 35.9 (100%)
Scopolamine[c]	15.0 ± 7.0 (20.5%)[##]
Scopolamine +	
RGCDP-1 60 mg	26.1 ± 15.3 (35.6%)
120 mg	28.1 ± 18.7 (38.4%)
240 mg	34.4 ± 12.4 (47.0%)[*]

[a]Mice received RGCDP-1 intraperitoneally sixty min before the training trial. After 30 min, scopolamine was injected subcutaneously to induce amnesia (2 mg/kg body weight). Twenty four hours after the training trial, the mice were again placed in the compartment. The latency time to step down the grid was measured. The values shown are the mean ± SD. The numbers in the parentheses are the percentages of the control value.
[b]Control means saline-treated control group.
[c]Scopolamine means scopolamine-treated group.
[##]Result differs significantly from the value of control group at $p < 0.01$.
[*]Result differs significantly from the value of scopolamine-treated group at $p < 0.05$.

Table 3. Anti-amnestic effect of Korean red ginseng and crude drug-combined prescription, RGCDP-2, on scopolamine-induced memory impairment in mice

Experimental treatment[a]	Step down latency(s) (% of control)
Control[b]	62.0 ± 28.5 (100%)
Scopolamine[c]	6.1 ± 4.2 (9.8%)[##]
Scopolamine +	
RGCDP-2 60 mg	8.0 ± 5.7 (12.9%)
120 mg	10.4 ± 6.0 (16.8%)
240 mg	11.6 ± 7.4 (18.7%)

[a]Mice received RGCDP-2 intraperitoneally sixty min before the training trial. The following procedure is the same as in Table 1.
[b]Saline-treated control group.
[c]Scopolamine-treated group.
[##]Significantly different from the value of control group at $p < 0.01$.

scopolamine to induce memory impairment in mice. The impairment was evaluated using passive avoidance test with or without treatment with RGCDPs.

On the basis of Korean folk prescription, "Chongmyongtang, we prepared RGCDP-1 that combines Korean red ginseng with the prescription. It was demonstrated that RGCDP-1 possesses anti-amnestic activity at a dose of 240 mg/kg, by prolonging the step-down latency time shortened by scopolamine (Table 2). Under the hypothesis that the activity of RGCDP-1 could be augmented by synergism with other crude drugs used for vital energy

Table 4. Anti-amnestic effect of Korean red ginseng and crude drug-combined prescription, RGCDP-3, on scopolamine-induced memory impairment in mice

Experimental treatment[a]	Step down latency(s) (% of control)
Control[b]	85.4 ± 40.3 (100%)
Scopolamine[c]	12.2 ± 7.5 (14.3%)[##]
Scopolamine +	
RGCDP-3 60 mg	54.6 ± 28.1 (63.9%)[**]
120 mg	60.2 ± 31.0 (70.5%)[**]
240 mg	64.3 ± 27.4 (75.3%)[**]

[a]Mice received RGCDP-3 intraperitoneally sixty min before the training trial. The following procedure is the same as in Table 1.
[b]Saline-treated control group.
[c]Scopolamine-treated group.
[##]Significantly different from the value of control group at $p < 0.01$.
[**]Result differs significantly from the value of scopolamine-treated group at $p < 0.01$.

enhancement, RGCDP-3 was prepared by combining "Chongmyongtang"-based RGCDP-1 with "Guibitang"-based RGCDP-2. Table 3 shows RGCDP-2 was not effective in increasing of the step-down latency time shortened by scopolamine. Table 4, however, shows that RGCDP-3 prolonged the shortened step-down latency at all doses tested (60, 120, 240 mg/kg), indicating synergistic action among constituents of RGCDP-1 and RGCDP-2 resulted in the more potent activity of RGCDP-3 than that of RGCDP-1 in the amnesia-induced mice (Tables 2 and 4).

From the above mentioned results, it may be concluded that the activity of crude drugs acting on brain function could be augmented by the synergistic effect of those with vital energy enhancing property.

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References

Bartus, R.T., Dean III, R.L., Beer, B., and Lippa, A.S., The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408-414 (1982).

Beatty, W.W., Butters, N., and Janowsky, D.S., Patterns of memory failure after scopolamine treatment: implications for cholinergic hypothesis of dementia. *Behaviour and Neural Biology* **45**, 196-211 (1986).

Christensen, H., Maltby, N., Jorm, A.F., Creasey, H., and Broe, G.A., Cholinergic "blockade" as a model of the cognitive deficits in Alzheimer's disease. *Brain* **115**, 1681-1699 (1992).

Fibiger H., Cholinergic mechanisms in learning, memory, and dementia: A review of recent evidence. *Trends in Neuroscience* **14**, 220-223 (1991).

Irie, Y. and Keung, W.M., Rhizoma acori graminei and its active principles protect PC-12 cells from the toxic effect of amyloid-β peptide. *Brain Research* **963**, 282-289 (2003).

Kim, S.R., Hwang, S.Y., Jang, Y.P., Park, M.J., Markelonis, G.J., Oh, T.H., and Kim, Y.C., Protopine from Corydalis ternate has anticholinesterase and anti-amnestic activities. *Planta Med.* **65**, 218-221 (1999).

Kopelman, M.D. and Corn, T.H., Cholinergic blockade as a model of cholinergic depletion. *Brain* **111**, 1079-1110 (1988).

Neri, M., Andermarcher, E., Pradelli, J.M., and Salvioli, G., Influence of a double blind pharmacological trial on two domains of well being in subjects with age associated memory impairment. *Arch. Gerontol. Geriatr.* **21**, 241-252 (1995).

Park, C.H., Choi, S.H., Koo, J.W., Seo, J.H., Kim, H.S., Jeong, S.J., and Suh Y.H., Novel cognitive improving and neuroprotective activities of *Polygala tennuifolia* Willdenow extract, BT-11. *J. of Neuroscience Research* **70**, 484-492 (2002).

Wen, H., Yoshimura, S., Matsuda, and Sakanaka, M., Ginseng root prevents learning disability and neuronal loss in gerbils with 5-minute forebrain ischemia. *Acta Neuropathol.* **91**, 15-22 (1996).

Wu, C.R., Hsieh, M.T., Huang, S.C., Peng, W.H., Chang, Y.S., and Chen, C.F., Effects of Gastrodia elata and its active constituents on scopolamine-induced amnesia in rats. *Planta Med.* **62**, 317-321 (1996).

Zhao, R. and McDaniel, W.F., Ginseng improves strategic learning by normal and brain-damaged rats. *NeuroReport* **9**, 1619-1624 (1998).

Zhong, Y.M., Nishijo, H., Uwano, T., Tamura, R., Kawanishi, K., and Ono, T., Red ginseng ameliorated place navigation deficits in young rats with hippocampal lesions and aged rats. *Physiol. Behav.* **69**, 511-525 (2000).

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