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Inhibitory Effects of the Korean Red Ginseng Extract on the Content of Neurotransmitter-Related Components of the Mouse Brain in Convulsion-induced Model

Jongwon Choi[1,*], Yeong-Min Yoo[2], and Hee-Juhn Park[2]

[1]*College of Pharmacy, Kyungsung University, Busan 608-736, Korea*
[2]*Division of Environmental Botany, Sangji University 220-702, Korea*

Abstract – Treatment of mice with Korean Red Ginseng (KRG) changed glutamic acid and GABA content in the mouse brain tissue with pentylenetetrazole (PTZ)-induced convulsion. KRG were orally administered at a dose of 50, 100 mg/kg for two weeks. The electroconvulsions (MES) and PTZ-induced convulsion were reduced but those induced by strychnine, bicuculine and picrotoxin were not. PTZ-induced convulsion decreased the ã-aminobutyric acid (GABA) content in brain compared to control group while the content was increased in KRG-treated group compared to PTZ group. In the PTZ-treated group, the GABA-transaminase (GABA-T) activity was increased by 59.6%, while no effect was observed on glutamate decarboxylase (GAD) activity. These results support that the KRG decreased the GABA contents and modulated the glutamic acid contents in the brain.
Keywords – Korean Red Ginseng (KRG), pentylenetetrazone (PTZ), GABA, GAD, GABA-T, glutamic acid

Introduction

Ginseng is a well-known medicinal herb and has been used traditionally in East Asia. As an adaptogen, *Panax ginseng* C.A. Meyer (Araliaceae), which grows in China and Korea, has a variety of biological actions; anti-carcinogenic, anti-diabetic and anti-inflammatory effects, cardiovascular protection and neuroprotection (Zhang, *et al*., 1966; Joo, *et al*., 2005). *P. ginseng* inhibits several types of cancers in animals and human (Shin, *et al*., 2000). As cancer is rapidly becoming the leading cause of death in the world, many reports have focused on chemoprevention trials with *P. ginseng*. Most of the pharmacological actions of ginseng are attributed to a variety of ginsenosides which are triterpenoid saponins (Attele, *et al*., 1999; Huang, *et al*., 2005). The physiological and pharmacological effects can be differing from a various ginsenosides and functions can even be oppositional (Sengupta, *et al*., 2004; Joo, *et al*., 2005). As ginsenosides have the opposite effects and a type of ginsenoside has multiple actions, the overall pharmacological functions of ginseng are complex. Ginseng extracts have been reported to be a wide range of biological actions (Nishino, *et al*., 2001).

About 1% of total population in the world and 2,500,000 people in United States have been suffered from epilepsy. Those statistical data in Korea was indefinite. The prevalence of this disease has increased up to recently. Epilepsy can be caused by hereditary factor, head trauma and brain capillary obstacle such as brain degenerative disease and brain infection (Kokenge, *et al*., 1965; Raines, *et al*., 1973). Furthermore, this disease may be found as cause of some accident including a traffic accident or an incident in workshop which can be stimulated by spotlight, or drug abuse, which have been reported to increase gradually. The convulsion occurred as a result of the excitability inhibition in the CNS, which can cause imbalance in spasm and this theory related to amino acid was broadly discussed (Crawford, 1963; Croucher, *et al*., 1982). Since phenobarbital was introduced in 1912, 20 drugs on epilepy have been discovered, which includes the experimental stage and controlled clinical trial stage; voltage-dependent ionic channel modulation, enhancement of GABA-mediated inhibition and suppression of acidic amino acid mediated excitation

*Author for correspondence
Fax: +82-51-620-4804; E-mail: jwchoi@ks.ac.kr

(Allen and Griffiths, 1984). But since a severe and intractable epilepsy patient was usually treated with two kinds of drugs which the full mechanism of antispasm was not understood clearly, the drug can cause several side effects such as sleepiness, headache, distraction, depression, impediment, and frequent incessant. That suggests why the development of a new drug for antiepilepsy is important (Berl, *et al*., 1961; Kokenge, *et al*., 1965; Raines, *et al*., 1973; Kingsley, *et al*., 1980).

Therefore, we performed the study to investigate KRG the anticonvulsant activities with MES, bicuculine and strychnine test. Moreover, we inquire into glutamic acid and GABA content in the mouse brain tissue with PTZ-induced convulsion that influenced by KRG.

Experimental

Animals – The ICR mice (20 ± 10 g) was purchased from Dae Han Bio Link Co., allowed to adapt to laboratory conditions (temperature: 20 ± 2 °C, dampness: 40 - 60%, light/dark cycle: 12 h) for a week. Animals were treated with Korean Red Ginseng powder (Korean Ginseng Corp.) for two week and 24 hours before the experiment, only water was offered to the animals. Each group was fed a normal AIN-76 purified diet (Central Lab. Animal Inc. Seoul). Considering the variation of enzyme activity, the animals were sacrificed at fixed time (10:00 A.M. - 12:00 P.M.). These experiments were approved by the University of KyungSung Animal Care and Use Committee. All procedures were conducted in accordance with the "Guide for Care and Use of Laboratory Animals" published by the National Institutes of Health.

Pharmacological seizure test – Electroconvulsions (MES) were produced by means of an alternating current (0.2 s stimulus duration, 60 Hz, 50 mA, 110 V) delivered via ear-clip electrodes by a generator (Hugo Basil, Italy). The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis). Strychnine seizure (Araki and Ueki, 1972) using strychnine (2.5 mg/kg, i.p.), pentylenetetrazole seizure (Sohn, *et al*., 1970) using pentylenetetrazole (70 mg/kg, s.c), bicuculine and picrotoxin seizure (Holland, *et al*., 1992) using bicuculline (3.2 mg/kg) and picrotoxin (5.0 mg/kg) were induced and animal's spasm and death were observed.

Enzyme preparation – Animals were anaesthetized using CO_2, the head was operated and incised in median line and brain tissue were taken and cleaned using 0.9% NaCl saline, 1 g tissue was added with 1 ml of 0.1 M potassium phosphate buffer (pH 7.5) and homogenized using glass-Teflon homogenizer. Homogenate was centrifuged at 35,000 × g for 30 min and the supernatant was analyzed.

Table 1. Conditions of HPLC for the determination of brain GABA and glutamic acid concentration in mouse

Parameter	Conditions
Column	RP-C_{18} (150 × 4.0 mm I.D., 10 μm)
Flow rate	0.6 ml/min
Mobile phase	10 mM potassium acetate buffer (pH 6.5) - Methanol
Gradient	Methanol 20% 70%/40 min
Attenuation	8
Detector	Fluorescence detector (λ_{Ex}: 340 nm, λ_{Em}: 450 nm)

Brain GABA and glutamic acid contents – GABA and glutamic acid contents was determined according to Allen and Griffiths (1984). Brain tissue was added with 1 mM aminoethylisothiouronium bromide and 2 mM pyridoxal-5'-phosphate containing 0.3 M triethanolamine buffer (pH 6.8) to make 10% homogenate, centrifuged at 15,000 × g for 20 min. Postmitochondria fixation was added with 200 mM potassium phosphate buffer (pH 6.8) and cold ethanol to remove the protein. After centrifuged, supernatant was filtered using membrane filter and the remaining filtrate containing GABA and glutamic acid was detected using HPLC with comparing the reference retention time (GABA: 11.3 min, glutamic acid: 19.8 min) (Table 1).

GABA transaminase (GABA-T) activity – Enzyme activity was determined according to Bergmeyer, *et al*. (1983) method. 0.15 M Potassium Phosphate buffer (pH 6.8) was added with 100 μl of 60 mM α-ketoglutaric acid, 50 μl of 4 μM GABA and 100 μl enzyme (100 **- 200 μg proteins) and** incubated for 30 min. Succinic semialdehyde and 10 μl of 0.12 mM NADP was added and reacted for 20 min. Absorbance was measured at 340 nm.

Glutamate decarboxylase (GAD) activity – Enzyme activity was determined according to Allen and Griffiths (1984), 200 mM potassium phosphate buffer (pH 6.8), tissue supernatant, 200 μl of 0.4 mM glutamic acid and 400 μl of 10 mM pyridoxal-5'-phosphate mixed with 100 μl enzyme (100 **- 200 μg proteins)** and incubated for 20 min and added with cold ethanol to remove protein and the centrifuged. The mixture was filtered using membrane filter and GABA was detected using HPLC.

Protein concentration and statistical analysis – Protein total was determined by method of Lowry, *et al*. (1951) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a standard. The results were

Table 2. Effect of Pretreated of Korean Red Ginseng (KRG) on the MES-induced convulsion and death in mice

Treatment	KRG (mg/kg, p.o)	N	Convulsion (%)		Death (%)
			TE	CL	
Control		10	100	20	80
KRG	50	10	100	20	80
	100	10	60	50	50
	200	10	60	60	40
MES	100	10	0	100	0

Mice were orally administered KRG daily for two weeks and a one hour after the final treatment of KRG; animals were received MES-induced seizure. The procedure was described in the experimental methods. TE, tonic extensive convulsion; CL, clonic convulsion.

analyzed and verified using Duncan's multiple range tests. Differences were considered significant as $p < 0.05$.

Results

Anticonvulsant effect of Korean Red Ginseng (KRG) against maximal electroshock-induced seizures in mice – Effect of KRG pretreatment on the MES-induced convulsion and death in mice was shown in Table 2. 50 mg/kg KRG did not inhibit the convulsion compared to control groups, but 100 and 200 mg/kg KRG suppressed. 50, 100 and 200 mg/kg of KRG for 2 weeks decreased convulsion and death rate.

Anticonvulsant effect of KRG against strychnine-induced seizures in mice – 50 and 100 mg/kg KRG on strychnine (2.5 mg/kg, i.p.) induced convulsion in mice was shown in Table 3. There is no significance difference between KRG-treated groups and control group in onset convulsion, tonic extensive convulsion or mortality of mice.

Anticonvulsant effect of KRG against bicuculine and picrotoxin-induced seizures in mice – When we examined the anticonvulsant effect of KRG in the bicuculine (2.7 mg/kg, i.p.) and picrotoxin (3.2 mg/kg, i.p.)-induced convultion model, as shown in Table 4 and 5.

The anticonvulsion test was used to determine whether KRG has toxic extensive convulsion and mortality in mice, and as expected KRG showed not significant activity.

Anticonvulsant effect of KRG against pentylenetetrazole (PTZ)-induced seizures in mice – The anticonvulsant effect of KRG in the PTZ (70 mg/kg, i.p)-induced convultion model, as shown in Table 6. KRG prolonged the onset increase, the latent incidence of convulsion and mortality.

KRG effect on the brain GABA and glutamic acid level in brain of PTZ-induced mice – Fifty and one hundred mg/kg KRG were pretreated for 2 weeks and induced with PTZ-induced convulsion model. In Table 7, the KRG-traeted group showed the inceasment in GABA contents and decreasment in glutamic acid compared to PTZ group.

Table 3. Influence of pretreated of KRG on strychnine induced convulsion and mortality in mice

	(mg/kg, p.o.)	N	Onset		TE		Mortality	
			Inc (%)	Lat (sec)	Inc (%)	Lat (sec)	Inc (%)	Lat (sec)
Control	0	10	100	225.1 ± 35.4^b	100	237.7 ± 25.8^a	100	261.4 ± 21.6^a
KRG	50	10	100	269.3 ± 18.2^b	100	298.8 ± 38.2^a	100	300.7 ± 33.5^a
	100	10	100	265.7 ± 17.5^b	90	300.3 ± 33.7^a	80	320.6 ± 43.4^a
Strychnine	100	10	90	474.2 ± 28.8^a	40	–	0	–

Values are mean ± SD (n = 10). Data followed by different superscript are statistically significant by Duncan's new multiple range test from normal ($p < 0.05$). TE, tonic extensive convulsion; Inc, incidence; Lat, latent time from strychnine treatments.

Table 4. Influence of pretreated of KRG on bicuculine-induced convulsion and mortality in mice

	(mg/kg, p.o.)	N	Onset		TE		Mortality	
			Inc (%)	Lat (sec)	Inc (%)	Lat (sec)	Inc (%)	Lat (sec)
Control	0	10	100	352.2 ± 45.2^b	100	373.8 ± 45.8^a	100	392.4 ± 31.6^a
KRG	50	10	100	379.3 ± 28.0^b	100	395.9 ± 28.5^a	100	423.2 ± 33.5^a
	100	10	100	394.9 ± 37.5^b	90	410.6 ± 33.3^a	80	440.1 ± 23.9^a
bicuculine	100	10	80	487.1 ± 48.8^a	30	–	0	–

Values are mean ± SD (n = 10). Data followed by different superscript are statistically significant by Duncan's new multiple range test from normal ($p < 0.05$). TE, tonic extensive convulsion; Inc, incidence; Lat, latent time from bicuculine treatments.

Table 5. Influence of pretreated of KRG on picrotoxin-induced convulsion and mortality in mice

	(mg/kg, p.o.)	N	Onset		T.E.		Mortality	
			Inc (%)	Lat (sec)	Inc (%)	Lat (sec)	Inc(%)	Lat (sec)
Control	0	10	100	615.0 ± 25.3^{b}	100	627.6 ± 35.7^{a}	100	651.3 ± 21.5^{a}
KRG	50	10	100	649.2 ± 38.1^{b}	90	665.6 ± 28.1^{a}	90	681.1 ± 33.4^{a}
	100	10	90	654.1 ± 27.4^{b}	80	685.5 ± 23.2^{a}	80	700.0 ± 38.9^{a}
picrotoxin	100	10	70	821.8 ± 48.5^{a}	40	–	0	–

Values are mean ± SD (n = 10). Data followed by different superscript are statistically significant by Duncan's new multiple range test from normal ($p < 0.05$). TE, tonic extensive convulsion; Inc, incidence; Lat, latent time from bicuculine treatments.

Table 6. Influence of pretreated of KRG on pentylenetetrazole (PTZ)-induced convulsion and mortality in mice

	(mg/kg, p.o.)	N	Onset		TE		Mortality	
			Inc (%)	Lat (sec)	Inc (%)	Lat (sec)	Inc (%)	Lat (sec)
Control	0	10	100	45.1 ± 5.14^{b}	100	159.3 ± 6.72^{b}	100	191.5 ± 5.88^{c}
KRG	50	10	100	54.2 ± 3.99^{b}	90	172.8 ± 8.25^{b}	90	221.6 ± 9.27^{b}
	100	10	80	83.7 ± 9.98^{a}	60	210.4 ± 11.9^{a}	40	289.8 ± 20.0^{a}
PTZ	100	10	0	–	0	–	0	–

Values are mean ± SD (n = 10). Data followed by different superscript are statistically significant by Duncan's new multiple range test from normal ($p < 0.05$). TE, tonic extensive convulsion; Inc, incidence; Lat, latent time from bicuculine treatments.

Table 7. Effect of pretreatment of KRG on the brain GABA and glutamic acid level in PTZ-induced mice

Treatment	Dose mg/kg	GABA Nmole/mg protein	Glutamic acid nmole/mg protein
Control		2.18 ± 0.23^{a}	$9.84 \pm 0.35^{c,d}$
KRG	50	$1.69 \pm 0.14^{c,d}$	$15.8 \pm 0.28^{a,b}$
	100	1.87 ± 0.19^{b}	12.7 ± 0.23^{c}
PTZ		1.30 ± 0.11^{d}	18.3 ± 0.51^{a}

KRG was administrated once a day for two weeks orally to mice. A one hour after the final treatment of KRG, animals were received pentylenetetrazole (70 mg/kg, i.p.). The procedure was described in the experimental methods. Values are Mean ± SD (n = 10) and values followed by the same superscript are not significant ($p < 0.05$) each other by new multiple square method.

KRG effect on the brain GABA and glutamic acid biosynthesis enzyme in PTZ-induced mice – KRG effect on GABA-T and GAD activity against PTZ-induced convulsion was shown in table 8. GABA-T activity of control group was 1.36 ± 0.08 NADPH nmole/mg protein/min whereas that in PTZ induced group was 2.17 ± 0.14 NADPH nmole/mg protein/min, which increases approximately 59.6%. Activities of 50 and 100 mg/kg KRG were 1.78 ± 0.18 and 1.62 ± 0.20 NADPH nmole/mg protein/min, whose rise is approximately 30.8% and 19.1%, respectively. There is no significance GAD activity among control group, PTZ-induced group and KGR group.

Table 8. Effect of pretreatment of KRG on the brain GABA-T and GAD activities in PTZ-induced mice

Treatment	Dose	GABA-T activity*	GAD activity**
Control		1.36 ± 0.08^{b}	7.32 ± 0.50 ns
KRG	50	1.78 ± 0.18^{b}	7.67 ± 0.51
	100	$1.62 \pm 0.20^{b,c}$	7.59 ± 0.39
PTZ		2.17 ± 0.14^{a}	7.88 ± 0.46

KRG was administrated once a day for two weeks orally to mice. A one hour after the final treatment of KRG, animals were received pentylenetetrazole (70 mg/kg, i.p.). The procedure was described in the experimental methods. Values are Mean ± SD (n = 10) and values followed by the same superscript are not significant ($p < 0.05$) each other by new multiple square method. Ns, not significant; *, NADPH nmole/mg protein/min; **, GABA nmole/mg protein/min.

Discussion

KRG effect on brain GABA/glutamic acid content and convulsion was investigated. The anticonvulsion effects of test KRG was assayed using two convulsion models, i.e. by the MES and PTZ test in mice. KPG showed anticonvulsion activity after oral administration of 50, 100 mg/kg. MES test generated the *generized tonic chlonic seizure* symptoms in gland mal whereas strychnine stimulated the CNS but mainly affected the spinal cord. Strychnine is a competitive antagonist on glycine receptors that cut off and block the conjugation (postsynaptic inhibition) and cause anchylosis. Bicuculine

and picrotoxin inhibit competitively $GABA_A$ receptor (presynaptic inhibition) in the CNS and cause convulsion (Swinyard, 1969; Bircher *et al*., 1962; Chapman *et al*., 1983). PTZ differs with strychnine and picrotoxin in the mechanism of conjugation in presynaps and postsynaps and affects the chloride channel of GABA receptor, causing no electrical charge and seizure or myochlonic seizure. Myochlonic seizure in animal model is used to find the drug acting on epilepsy or paroxysm disease in animal model (Metcalf, 1979; Holdiness, 1983). GABA-T is found in the brain tissue which catalyzed the transamination of GABA to succinic semialdehyde (Dulac and Arthuis, 1984; Elwes *et al*., 1984). GABA-T activity of PTZ-treated group was increased approximately 59.6% and that of KRG-treated group was decreased, compared to control group, respectively. It means that KRG can change GABA content in the brain via GABA-T activity. On the other hand, the PTZ-treated group was increased in glutamic acid content whereas administration of KRG was decreased. It appears that KRG did not modulate the glutamic acid contents on the PTZ-induced convulsion. The increment of glutamic acid content can be caused by its increase in the GABAergic neuron when GABA-T activity and glutamicnergic neuron activity increased (Dulac *et al*., 1991; Sachdeo *et al.,* 1992). But glutamic acid content and GABA-T activity of PTZ-treatment group were increased. It appears that glutamic acid intake or biosynthesis catalyzed by GAD increasing GABA in the brain tissue (Leppik *et al*., 1993; Shorvon, 1991; Kohn *et al*., 1991; Hays *et al*., 1994).

As phenytoin have been used to treat epilepsy and have side effects of idiosyncratic or tolerance, the research to develop a new drug have been investigated in natural resources. The diagnosis of epilepsy bases on the brain wave imaging and shows the abnormality in nerves electrical discharge. Especially, CNS innervation through the imbalance of glutamic acid and GABA as a neurotransmitter that can cause spasm attack was indicated, and since 1980 the researches based on this neurotransmitter have been developed. Glutamic acid from dietary intake is a source for GABA biosynthesis catalyzed by GAD. In mammals, GABA is found in high concentration in the brain and spinal cord, hyperpolarizing CNS and depolarizing the synapse of the CNS. In the brain tissue, GABA is biosynthesized from L-glutamic acid through decarboxylation by GAD, whereas glutamic acid can be formed from á-ketoglutarate by transamination in process of TCA cycle. It is also synthesized from putrescine in the liver, kidney, spleen and lungs. GABA will be metabolized to glutamic acid and succinic semialdehyde by GABA-T (Curtis and Johnston, 1974; Enna *et al*., 1980). In vertebrates, there are two major types of GABA receptors, the ionotropic $GABA_A$ and the metabotropic $GABA_B$ receptor. $GABA_A$ receptor can be modulated by muscimol, isoguvacine, bicuculine and an antagonist competitive picrotoxin. Benzodiazepine, an agonist of $GABA_A$ receptor, coupled to increase chloride conductance. On the other hand, $GABA_B$ receptor, a superfamily of G-protein-coupled receptors, has selective affinity to baclofen and its lack of affinity for muscimol and bicuculine. Unlike $GABA_A$ receptor that directly associated with chloride channel, $GABA_B$ receptor seemed to be coupled to calcium or potassium channels via adenylate cyclase (McGeer *et al*., 1987; Sieghart, 1989; Bowery, 1989). The balanced function of amino acid in the CNS tissue was affected by biosynthesis, receptor binding, reuptake and metabolism. In the PTZ-treatment group, glutamic acid content was increased, following the increased activity of GAD. On the other hand, this change was not observed in KRG-treated group, which means that KRG regulate the GABA contents without GAD activity. This result shows that KRG affects the convulsion electric charge in brain tissue, GABA and glutamic acid contents, and then GABA-T activity induced. This also may suggest that KRG action in the increment of GABA level is related to GABA-T activity.

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