Efect of Herbal Medicinal Preparations Containing Ginseng on Learning and Memory in Kainate-induced Seizures

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Abstract

Panax ginseng and the herbal medicinal mixtures containing ginseng have been widely used as a traditional medicinal prescriptions. In order to develop more efficient and protective prescriptions on seizures and subsequent memory deterioration, we investigated the biochemical and ethopharmacological effects of ginsenosides and fractions from the natural medicinal plant products related to control convulsions. In this studies we show results improving spatial learning and memory deficits induced by kainic acid, a potent neurotoxic and neuroexcitatory analogue of the amino acid neurotransmitter glutamate.

Introduction

Convulsions and seizures during infancy could bring about the results such as an obstruction of central nervous system including neurochemical changes, increase of seizure susceptibility and impediment of cognitive functions as a sign over the long periods. Kainic acid (KA), an analogue of the naturally occurring excitatory amino acid neurotransmitter glutamate, when given

either systemically or intracerebrally to adult rats, results in a condition similar to human temporal lobe epilepsy (Nadler *et al.*, 1980; Ben-Ari *et al.*, 1985). KA-induced seizures result from neuronal damage, especially in the hippocampus and amygdala complex.

It has been known that there were no pathological changes in immature brain after a single KA-induced seizures unlike in adult rats (Pollard *et al.*, 1994) although KA is sufficient to induce status epilepticus in immature rats with comparatively lower dosage than adults. The mechanism underlying the age-dependent selective vulnerability of the developing brain to the consequences of early seizures is not yet clarified. However, it may possible to study seizure activity itself and actions with compounds so called AED (Anti-Epileptic Drug) related to GABAergic metabolism such as vinyl-GABA (v-G), an irreversible inhibitor of gamma-amino-butyric acid transaminase (GABA-T) since GABA (-amino butyric acid) is a major inhibitory neurotransmitter in mammalian Central Nervous System (CNS; Cooper, *et al.*, 1986). The release of GABA by nerve terminals and its subsequent binding to its receptor must be followed by a rapid inactivation of the neurotransmitter.

When the concentration of GABA in brain diminishes to below a threshold level, various neurological disorders including epilepsy, seizures, convulsions, Huntingtons disease, and parkinsonism may occur (Perry *et al.*, 1973; De Biase *et al.*, 1991; Lloyd *et al.*, 1997). The concentration of GABA in the brain is controlled by two GABA shunt enzymes, glutamate decarboxylase (GAD) and GABA-T. The first enzyme catalyzes the synthesis of GABA, whereas the second enzyme catalyzes the conversion of GABA to succinic semialdehyde. The activation of GAD or the inactivation of the GABA-T in brain tissues increases the concentration of GABA.

It has been postulated that the regulation of GABAergic neurotransmission may be an important action of some ginsenosides which were purified from the root of *Panax ginseng C.A. Meyer* (James *et al.*, 1982; Kimura *et al.*,

1994). Our previous results showed that diol ginsenosides such as Rb2 and Rc increased GAD activities in a dose-dependent manner *in vitro* in response to increasing concentrations of Rc (Choi *et al*, 1994, 1998). Among the GABA shunt enzymes only the GAD activities were increased after total ginsenosides and PD saponins treatment in vivo (Choi *et al*, 1998).

We studied the effect of ginsenosides on KA-induced seizure activities associated with the influence of Rc regarding GAD enzyme activation. Especially, we examined the level of PKC-isozymes related to the reduction of seizure activities in the hippocampus of KA-injected immature rats.

On the other hand, we screened components from the natural plants which may control the enzymes of GABA shunt. In the combination studies we have obtained a new herb mixtures containing red ginseng saponins on the basis of the screen results, and examined the effects on the spatial learning and memory deficits induced by the kainic acid (KA) injection in Morris water maze.

1. GINSENG SAPONINS/ GINSENOSIDES

1.1 NORMAL AND SCOPOLAMINE-INDUCED LEARNING DISABILITY MODEL:

The effects of ginseng saponins having a different ratio of protopanaxadiol (PD) and protopanaxatriol saponins (PT) on the learning impairment induced by scopolamine, and learning and memory in mice were investigated in a passive avoidance task and a Morris water maze task. The ratio of PD and PT was 1.24 and 1.46, respectively.

Before training, the ginseng saponins improved the scopolamine-induced learning impairment at different dosages in mice, 50 and 100mg/kg, respectively. However, the two saponins did not show a faborable effect on

learning and memory in normal mice.

Korean red ginseng saponin with a low PD/PT ratio had an improving effect on spatial working memory, but the saponin with a high PD/PT ratio did not. This finding suggests that the PD/PT ratio of the ginseng saponins may be an important factor in the pharmacological role of red ginseng as a medicinal herb.

1.2 INFLUENCE OF GINSENOSIDES ON THE KAINIC ACID-INDUCED SEIZURE ACTIVITY IN THE IMMATURE RATS:

We studied the effects of ginsenosides in the immature rats based upon the previous results that ginseng has a suppressive or anticonvulsive activity. To examine the suppressive effect of ginsenosides on the kainic acid-induced seizures, the severities and frequencies were observed for 4 hr after injection of kainic acid (KA; i.p., 2 mg/kg b.w.) using 10 day-old male SD rats (22± 2g). Protopanaxadiol saponins such as ginsenoside-Rb1 (Rb1), ginsenoside-Rb2 (Rb2), ginsenoside-Rc (Rc) and ginsenoside-Rd (Rd) generally reduced the seizure activities while protopanaxatriol saponins such as ginsenoside-Rg1 (Rg1) and ginsenoside-Re (Re) rather increased stereotypic "paddling-like" movement. KA-induced seizure severities when vinyl-GABA (v-G) was injected together with Rb1 or Rc were additionally reduced only by the injection of Rc, but not by Rb1. The level of gamma isozyme of protein kinase C (PKC-γ) in hippocampus increased about 3 times as much as that of normal at 4 hr after KA injection. The increased level of PKC-γ by KA was significantly reduced to about 35% by the coinjection of v-G alone but it was not changed by v-G together with Rb1 or Rc. The increased level of PKC-γ at 4hr after injection of KA was not consistent with the reduction of seizure severities between Rb1 and Rc. These results suggest that Rc and Rb1 may reduce the seizure-severities independently of PKC-γ level, and Rc may additionally act with v-G regarding the GABA metabolism during the stage of KA-induced seizures in the immature rats.

2. GINSENG CONTAINING HERBAL MEDICINAL PREPARATIONS

2.1 EFFECT OF HERB MIXTURES ON LEARNING AND MEMORY AFTER KAINIC ACID-INDUCED SEIZURE IN IMMATURE AND ADULT RATS:

The effects of ginsenosides ratio on the facilitation of learning and memory may important in brain-damaged models as well as in normals. Learning impairment induced by scopolamine in a passive avoidance task and a Morris water maze task showed that ginseng saponin with a low ratio of protopanaxadiol to protopanaxatriol (PD/PT ratio) improved spatial working memory. However, PD-ginsenosides were more effective in an improvement of the spatial learning and memory deficits in kainate-induced seizures.

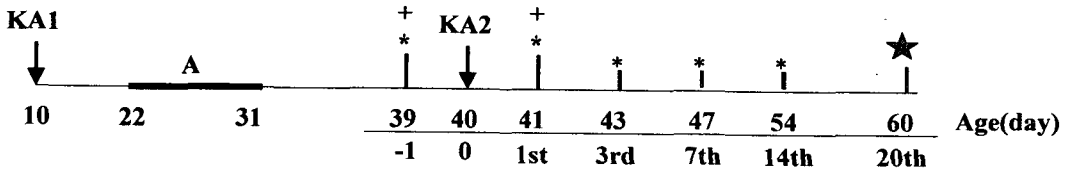
In order to investigate the ethopharmacological effects of GRC (Glutamate Regulating Components, herb components containing ginsenosides) on the spatial learning and memory deficits by the kainic acid (KA), reference and working memory in Morris water maze were tested after the KA treatment to 10 and 40 day-old rats. Acquisitions and probe trials were applied to the rats during 22-31 days old a single administration of the first KA (2mg/kg, i.p.) at 10 days old, and also compared with before and after the 2nd KA (8mg/kg, i.p.) injection at 40 days old until getting to the 60th days of age.

As results, it was observed that the experimental animals, which received KA at both 10th and 40th days old, were more susceptible to damage than the groups received KA at 40 days old in their spatial memory function. The working memory deficiency was not significantly ameliorated in GRC treated groups after the 2nd KA injection until the 3rd and the 7th day but did at the 14th day, and the reference memory deficit was also improved and kept significantly until the 20th day after the 2nd KA injection as compared with that of KA control ($p < 0.05$).

These results suggest that GRC may protect from not only the initial seizure severity but also ameliorate the long term impediments of spetial learning and memory caused by KA.

Scheme 1.

Time schedules after kainic acid injection for the memory test in Morris water maze



KA1; the 1st injection of kainic acid (2mg/kg i. p., at 10 days of age)
KA2; the 2nd injection of kainic acid (8mg/kg i. p., at 40 days of age)
A: acquisition training ; from 22nd to 31 days old, after KA1
Rats were swum two times a day (one time/a.m. and p.m. , r espectively)
* : probe trial (working memory) tests; KA2 -before, KA2-after
; one time/day at the 1st, 3rd, 7th and 14th day, respectivel y
+ reference memory (7 times/day) test; one day before or one d ay after KA2 injection,
★ ; reference memory (4 times a day) test at the 20th day (60days of old) after kA2

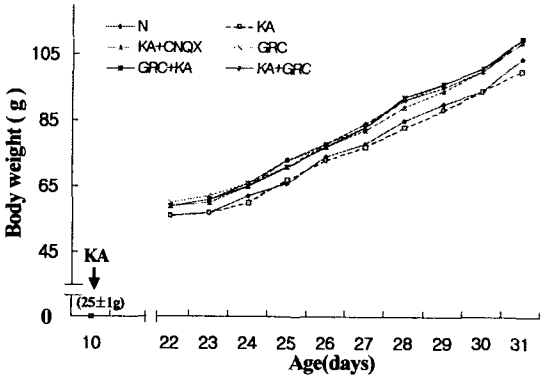


Fig. 1 Body weight change during the acquisition test after the 1st kainic acid injection

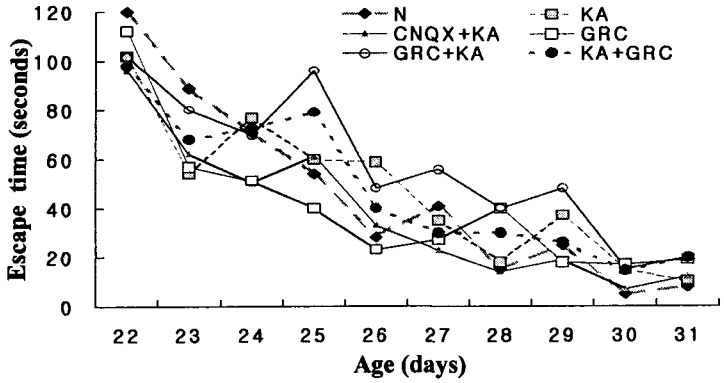


Fig. 2. Escape time during acquisition test after the 1st kainic acid injection

Rats received kainic acid (2mg/kg, i.p) at 10-day-old were trained in Morris maze test from 21 to 31 days old. The limit time was 120sec and data were expressed as mean± SE of each day (two trials a day).

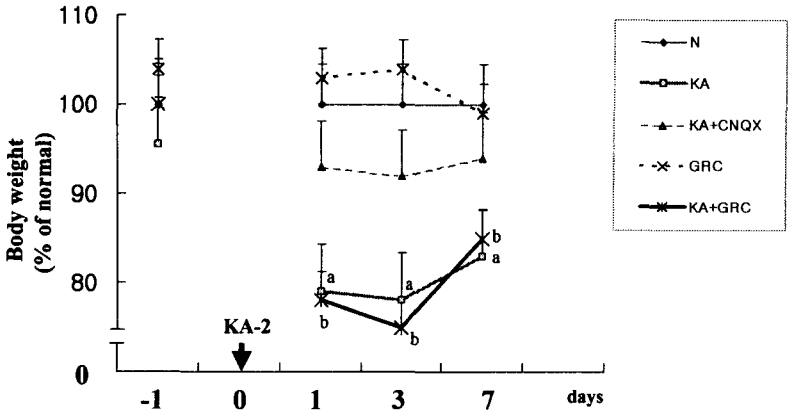


Fig. 3. Body weight changes after the 2nd kainic acid injection

Kainic acid (the 1st: 2mg/kg, and the 2nd: 8mg/kg, b.w.) was injected intraperitoneally.

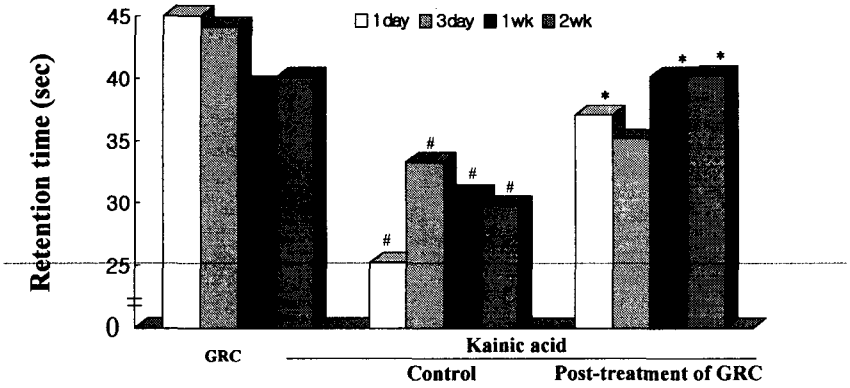


Fig. 4. Effect of GRC on retention time in the probe trials after the 2nd kainic acid injection

#: $p < 0.05$ normal vs kainic acid control, *: $p < 0.05$ kainic acid control vs [a]; KA+ GRC

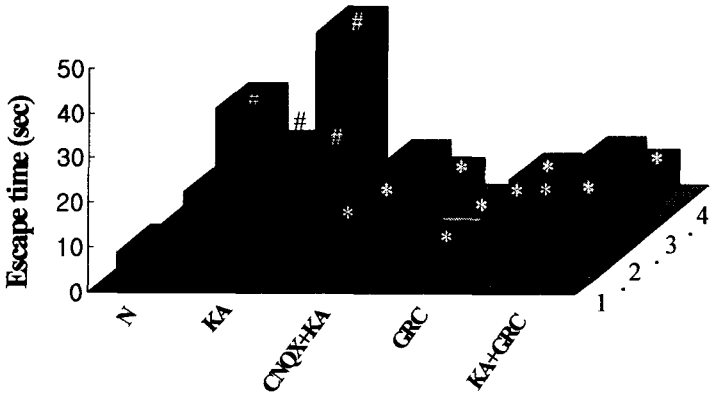


Fig. 5. Escape time at 20th day after the 2nd kainic acid injection

#; $p<0.05$ vs N *; $p<0.05$ vs KA, N; normal, KA; Kainic acid, CNQX+KA; KA after CNQX, GRC; red ginseng herb mixture fraction, A+GRC; GRC after Kainic acid (8mg/kg b.w.) injection.

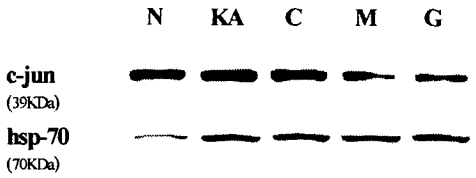


Fig. 6. Immunoblot analysis I; Effect of GRC on c-jun and hsp-70 in the immature rat hippocampus at 24hrs after systemic injection of kainic acid. N; normal, KA; kainic acid, C; CNQX, M; MK-801, G; GRC

Target	Normal	Kainic acid(KA)			
		Control	*CNQX*	*MK-801*	*GRC*
c-jun	99.9±5.4	120.6± 7.4#	76.3± 33.6	43.5 ± 9.0*	72.7± 22.9*
hsp-70	100.0 ±0.9	165.3±10.7#	113.2± 3.0*	146.9± 12.8	134.4± 11.6*

$p<0.05$ vs Normal(#), vs KA control(*)

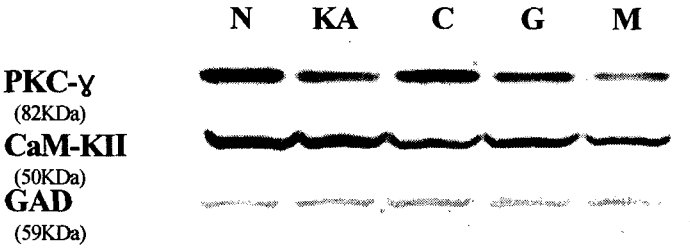


Fig. 7. Immunoblot analysis II; Effect of GRC on PKC-γ, CaM-KII and GAD in the immature rat hippocampus at 24hrs after systemic injection of kainic acid. N; normal, KA; kainic acid, C; KA+CNQX, M;KA+ MK-801, G;KA+GRC

Target	Normal	Kainic acid(KA)			
		Control	CNQX	GRC	MK-801
PKC-γ	100.0±4 .0	60.8±0.5#	100.0± 8.3*	85.1± 3.4*	61.0± 7.3
CaM-KII	100.0 ±1.9	89.7±11.7	86.3± 0.8	90.0± 0.6	84.3± 1.5
GAD	99.8 ± 10.1	89.9±17.9	121.6± 26.5	88.9±39.5	111.8±13.0

$p < 0.05$ vs Normal (#), vs KA control (*)

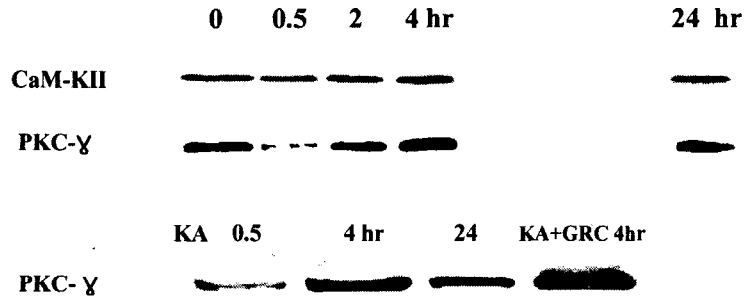


Fig. 8. Immunoblot analysis III; Effect of GRC on PKC-γ, CaM-KII in the immature rat hippocampus at 4 and 24hrs after systemic injection of kainic acid.

Conclusion

- GRC reduced the severity of seizure induced by the 1st kainic acid into the level of CNQX.
- GRC significantly ameliorated working memory deficit in the kainic acid-injected rat.
- GRC may normalize KA-induced neuronal damage by controlling GABA shunt enzymes, and PKC-γ in the seizure and may affect the long term impediment of learning and memory caused by kainic acid.

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