# Ginsenosides in the Mechanisms of Brain Excitability Regulation the Way for Progress

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## Introduction

The fundamental research of bioactive substances from Korean Red Ginseng (KRG) is necessary since the trust to phytotherapy among population in developing countries is growing. Researchers are looking for new phyto medicines and trying to improve the old ones for phytotherapy, neurology, psychiatry, oncology etc. It has been announced that 33 % of patients in USA are taking phyto medicines. 60 millions of Americans in 1997 used herbs for medical reasons [2, 21].

The prevention and treatment of epilepsy, multiple sclerosis, different problems of memory and cognitive functions are the crucial tasks of modern medicine. As the result of studies that were held by scientists of Lomonosov Moscow State University (Russia) and Korean Ginseng & Tobacco Research Institute (Korea) the antiepileptic properties of KRG and different extracts of ginseng were discovered. Inhibitory properties of ginseng compounds on the development of seizures were shown after intranasal administration in different models of epilepsy. At the same time the effectiveness of ginseng on spatial memory, cognitive functions and experimental amnesia improvement after severe epileptic strikes were established *[Jin-Kyu Park et al., 1995-2002]* [4,5, 22, 23]. The positive effect of KRG components on the process of postnatal development of animal brain (in the experiments from the neonatal age up to the maturity) was demonstrated. This testifies the possibility of large applied application of ginseng in some neurological diseases in children.

Phytotherapy in neurological diseases and in particular in epilepsy [2, 20, 21, 24] are being widely used, although the modern knowledge about the excitatory (glutamate) and inhibitory (GABA) transmitter systems allowed to develop new pharmacological drugs to control epilepsy in a very good way. However, over 20% of patients are resistant to any treatment in generally accepted way. Thus, the study of some plants properties can be a very solid base for further

implementation of traditional Eastern medicine where ginseng plays a leading role [6].

In 1994/1995 in cooperation with laboratory of Dr. Jin-Kyu Park we have collected experimental data concerning the antiepileptic properties of components of ginseng extracts and some definite ginsenosides (Rb1, Rg1, Rc). It was shown in experimental models of epilepsy in animals. At first we use the model of child febrile convulsions (heating-induced seizures in rat pups). The decrease of severity or complete inhibition of fits by ginsenosides was observed [4-6, 9-10, 16-19]. Further fundamental research showed the following physiological actions of ginsenosides: antioxidant properties, cytoprotection, neuroprotection, calcium channel inhibition [25], inhibition of reuptake of neurotransmitters, modulation of specific binding of $GABA_A$ and $GABA_B$ receptors. The total ginsenoside (n-butanol extract) fraction inhibited the uptake of radioactive GABA, glutamate, dopamine, noradrenaline and serotonin [28].

The aim of this study was an extension of research of antiepileptic properties of ginsenosides on the different kind of experimental models of epilepsy.

## Methods and Materials

The callus cell culture DAN25 from ginseng root was used [15, 25]. The ethanol and butanol extracts and lyophilic substances of ginsenosides [15, 16]were conducted in Joint-stock association ("Biokhimmash"). The content of extracts was evaluated (**Rg1+Re, Rf, Rb1, Rc, Rb2, Rd**); ginsenosides -7.552 mg/g - biomass of the extract.

The physiological effects were studied followed the Preclinical Anticonvulsant Screening Project proposed in USA. The following methods and experimental models were used:

- study of severity and threshold of seizures induced by chemoconvulsants (penthylentetrazole);
- audiogenic seizures in KM strain of rats;
- the measuring of cerebral blood flow (by hydrogen test modified by Dr. V.I. Sergeev in Lomonosov MSU), blood flow was registered by method of hydrogen clearance definition under the ethyl ether narcosis;
- EEG registration of spontaneously occurring spike-and-wave discharges in WAG/Rij rats with genetically determined absence epilepsy;
- EEG registration of pathological activity within cobalt cortical focus;
- ginsenosides was intracerebro-ventricularly (i.c.v.), intraperitoneally (I.P.) and orally administered.

As chemoconvulsant penthylentetrazole (PTZ) was used. The motor seizures severity in rats

were evaluated by using five-score scale described by P. Mares, H. Kubova [29]: **0**-no changes in behavior; **0.5**-atypical behavior (e.g. intensive grooming, sniffing, moving arrests); **1**-isolated mioclonic jerks, ear and facial twitching; **2**-atypical minimal seizures, convulsive wave through the body; **3**-fully developed minimal seizures, clonus of the head muscles and forelimbs, righting reflex was present; **4**-major seizures (generalized without the tonic phase); **5**-generalized tonic-clonic seizures, began with running followed by the lost of righting ability, then short tonic phase (flexion or extension of fore and hind limbs) progressed to the clonus of all four limbs.

The antiepileptic effects of ginsenosides complex from ginseng root cells were compared with the influence of chemically pure ginsenosides (Rg1, Rb1, Rc) and well known antiepileptic drugs - carbamazepine, valproic acid, lamictal.

All electrophysiological experiments were carried out in free-moving animals. EEG signals were filtered and recorded by CONAN software. The EEG recording was started immediately after the microinjections and was made for 4 hours. Number, mean duration and total duration of spontaneous spike-wave discharges were measured before and after ginsenosides administration.

## Results

In rats with audiogenic epilepsy (KM rat strain) the effectiveness of intraperitoneal administration of ginsenosides was shown (Fig. 1). The latency of seizures of 3$^{rd}$ and 4$^{th}$ score was signifi-

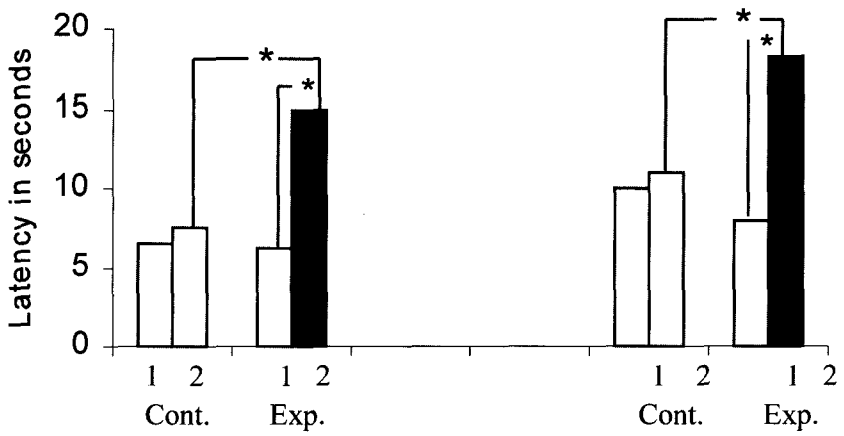


**Fig. 1.** Increasing of the latency of audiogenic seizures 3rd (left) and 4th (right) scores by ginsenosides in KM rats (in each group n=9). Control administration (I.P.) of saline-white bars, ginsenosides (5 mg/rat) administration-black bars [*-$p<0.01$]. The time between 1st and 2nd experiments was 12 days.

cantly increased. It means that the seizure threshold to the sound was enlarged. That was significant when we compared experimental and control (saline) groups, as well as when we compared the results in experimental group before and after administration of ginsenosides. The interval between 1st and 2nd experiment (1-2-Fig. 1) was 12 days.

The experiments on the PTZ model of convulsive epliepsy also proved the antiepileptic action of ginsenosides. The intraperitoneal administration of ginsenosides (5-7 mg/rat) induced the increasing of latency of clonic-tonic phase of seizures (Fig. 2A(2)). The duration of seizure response was significantly decreased (Fig. 2B). Thus, we can say that ginsenosides inhibited motor seizures even in the severe PTZ model of epilepsy (PTZ induces seizures potentiation by GABA A receptor).

The influence of ginsenosides on the brain blood flow (BBF) was investigated in rats by M.O. Pereversev. The baseline value of BBF without administration of any drugs except pentobarbital was conducted for several hours. It was shown that the reduction of velocity of BBF during the process of registration (2-3 h) was changed not more than 4% of baseline values, i.e. was stable. The systemic administration of ginsenosides caused the stabilization of BBF. The effect can be registered 15 min. after administration and can be retained for 30 min. In comparison with intraperitoneal administration, the intracerebral administration of ginsenosides significantly lowers the BBF and it is proven by 4 hours of observation. Two days later the experiment was repeated with the administration of ginsenosides in the same dosage after PTZ administration in the threshold dose (50 mg/kg). Less lowering of blood flow was observed. Possibly, it was caused by PTZ administration.

Thus, the preliminary study of the influence of ginsenosides on the blood flow in the rat brain on the background of PTZ administration allows to conclude that it has stabilizing effect on the seizure over susceptible brain. This data allows us to propose that combination of ginsenosides with other antiepileptic drugs is able to block their side effects on the BBF.

Neuronal mechanism of blood flow regulation in cerebral cortex include the transmitter regulation (noradrenaline, acetylcholine) and regulation by neuropeptides (Sub P, other tachykinins, NPY, CGRP etc.). "Metabolic sygnals" also responsible for the vasodilatation brain vessels. C. Iadecola et al. [11] demonstrated the role of activation of glutamate receptors, that neuronal increases in flow are linked to glutamate-induced depolarization of Purkinje cells and interneurons in cerebral cortex. We used the PTZ-induced seizures in the animal models of epilepsy. In humans it was shown that cortical blood flow (CBF) measured with $^{15}$0-water positron emission

tomography (PET) was increased asymmetrically. Only the patients with complex partial seizures had bitemporal 70-80% increase of CBF induced by PTZ administration [27]. These author demonstrated very important regularity: no significant correlation was noted between valproate level and CBF/ Valproate reduced regional CBF in human thalamus [27]. Evidence for the ginsenosides application in epileptology is the set of properties of ginsenosides both as the anticonvulsant drugs and regulators of CBF.

In a special series of experiments, performed by E. Tolmacheva, it was shown that the effects of ginsenosides as adaptogens have individual characteristics during the chronicle epileptical fits. Based on the method that was developed by L. Gorkavy at al. [12] we have researched the effects of ginsenosides on the condition of white blood. L. Gorkavy at al. have shown that the percentage of lymphocytes from leukocytes is an indicator of adaptive reactions of organism. It has shown that ginsenosides administration increases the number of lymphocytes [12]. And if the epileptic seizures in these rats are induced by PTZ administration the severity of epileptic response correlates with the functional condition of white blood. After ginsenosides administration in the group of rats where the level of activation of lymphocytes was 72-80% there was practically no tonic-clonic seizures (Fig. 3, A2). in the same group of rats that had developed epilepsy the latent period of tonic-clonic phase was three times higher (Fig. 3, A2). the same results we got when we used the root extract from *Eleutherococcus senticosus* (Fig. 3, A, B 3). These results show the correlation between the nonspecific adaptation reaction of organism and seizures readiness.

The improving, effective action of ginsenosides was demonstrated on the model of electrical kindling of amygdala in WAG/Rij rats with genetically determined absences (van Luijtelaar,

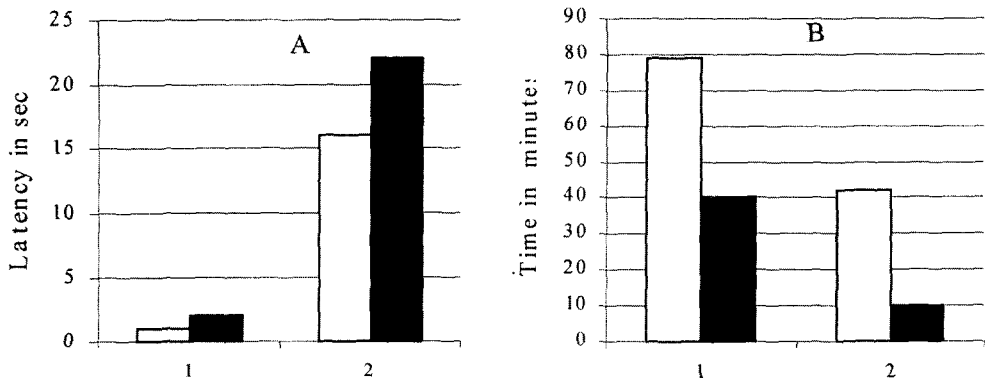


**Fig. 2.** A: Increasing of the latency of PTZ-induced (50 mg/kg, I.P.) seizures in rats by ginsenosides (5 mg/rat, I.P) B: Shortening of duration of PTZ-induced seizures in rats by ginsenosides. [* - P<0.05].

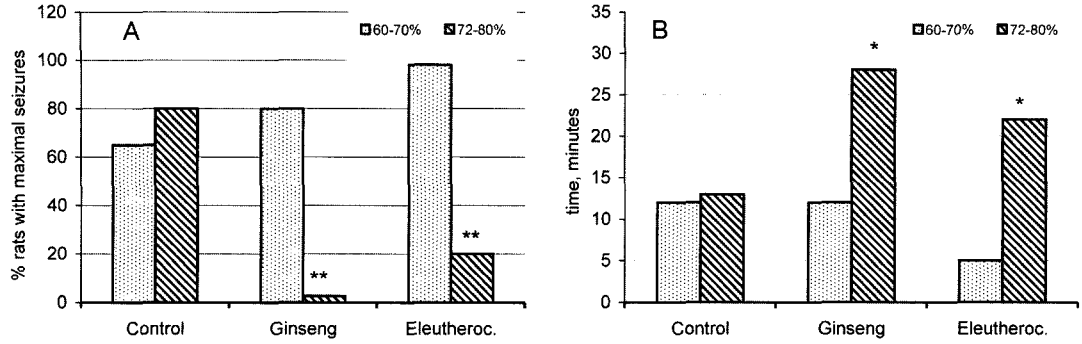


**Fig. 3.** Increase of the brain seizures threshold and the lymphocytes level by ginsenosides in rats.
A: The influence of ginsenosides from callus cell culture of ginseng -5 mg/rat and 6% extract of arallia (*Eleutherococcus senticosus*) on the seizures severity and lymphocytes level (in %) in rats (n=15).
B: The influences of ginsenosides and arallia on the latency of PTZ-induced generalized tonic-clonic seizures in rats. Datted bars-response of rats with lymphocyte level 60-70%, strocked bars-response of rats with lymphocyte level 72-80% (*-$p<0.05$; F-criteria; **-$p<0.001$; U-criteria).

Coenen, 1986 [8, 26]).

The influences of ginsenosides on the spontaneous spike-wave discharges (SWD) was investigated. I.c.v. administration of ginsenosides caused the significant changes in the pattern of SWDs was shown by the method of cortical EEG registration in WAG/Rij rats. EEG recordings were performed for 4 hours. The results are presented (Fig. 4). The columns show the absence period, the size of which represents the duration of absence discharges (SWD). The control administration of artificial cerebrospinal fluids didnt change the periodic character of absence (Fig. 4,-A). After i.c.v. administration of ginsenosides the practically full inhibition of spontaneous SWD was demonstrated one-hour later (Fig. 4,-B). Since the mechanism of genetically determined absence is drastically different from other kinds of epilepsy we have to continue studying this phenomenon in WAG/Rji.

Reduction of seizure frequency by ginsenosides during cobalt experimental epilepsy was studied in our laboratory by Melekhov M.G. Cobalt blocked the calcium channels in neuron membrane. Cobalt induced epilepsy is caused by changing the strength of recurrent inhibition in the cortex neurons [14]. The unilateral cortical cannula with cobalt powder was implanted simultaneously with the set of cortical electrodes for continuous recording of EEG. Ginsenosides (5 mg/rat) was administered intraperitoneally on the 21$^{st}$ day after cobalt implantation when the primary epileptic focus has been formed. We could not demonstrate a complete suppression of generalized seizure activity but the inhibition was more effective in the contralateral cortex in the sec-

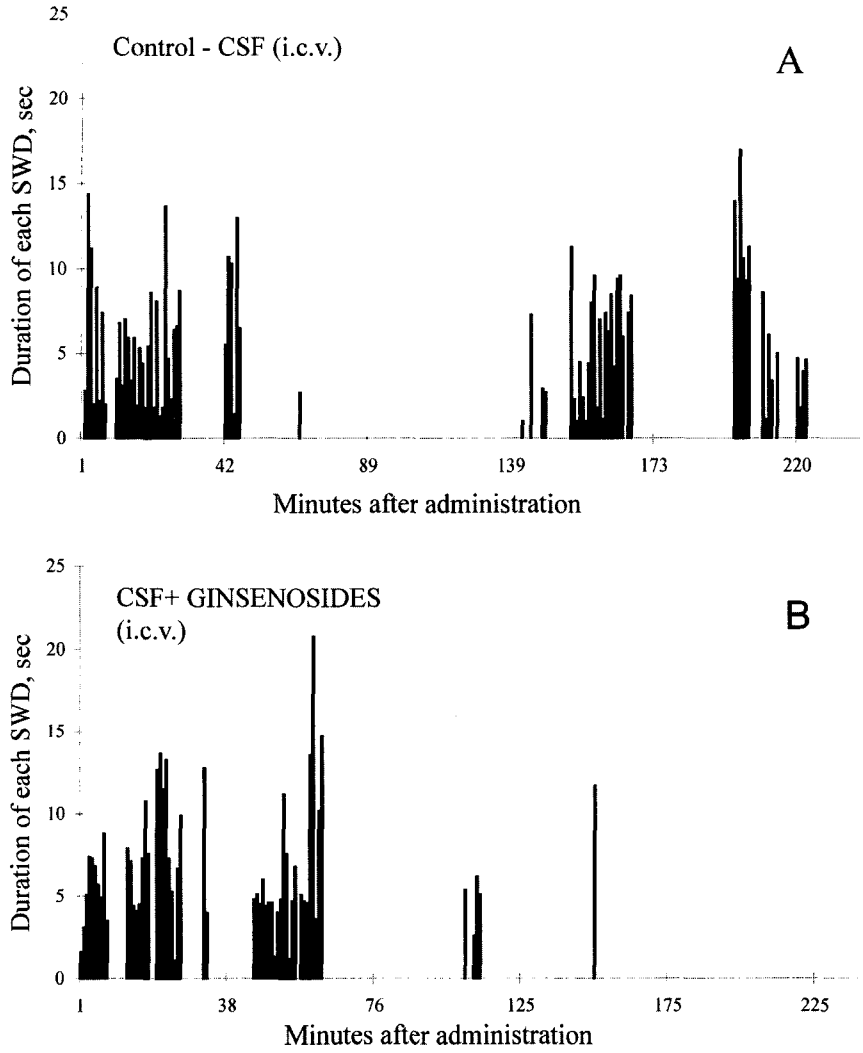


**Fig. 4.** Decrease of the number of spike-wave discharges (SWD) by ginsenosides (i.c.v.) in WAG/Rij rats with genetically determined absences A: after i.c.v. artificial CSF administration B: after i.c.v. administration of artificial CSF+ginsenosides (28.8 μg per rat).

ondary focus (Fig. 5).

It was demonstrated that GS is more effective in inhibition of transcortical generalization of seizures. Our results and data of B.Colasanti and C.Craig [10] suggest that the cobalt model of epilepsy may be useful in the study of mechanisms underlying both anticonvulsant effectiveness and rebound excitability after anticonvulsant drug withdrawal.

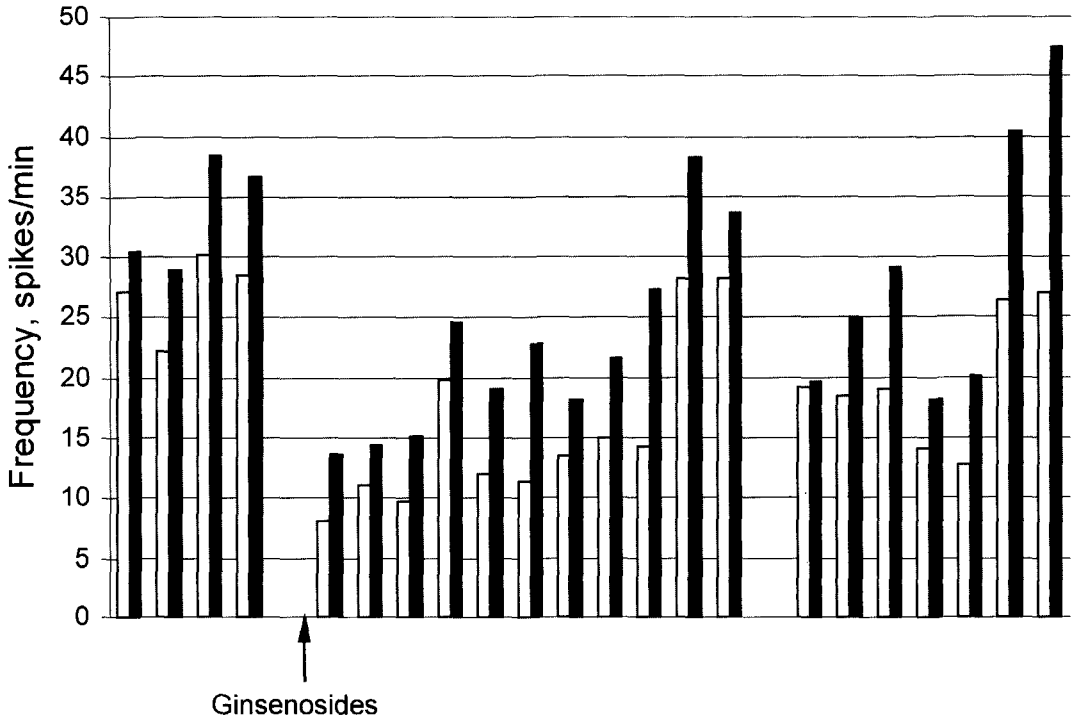


**Fig. 5.** Influence of ginsenosides on the primary and secondary cobalt cortical focus activity in Wistar rats. Black bars spike frequency in primary focus with cobalt, white bars-spike frequency in secondary focus of contralateral hemisphere.

## Discussion

In the mechanism of brain excitability regulation should involved the modulation of GABAergic transmission, that must be the important action of some ginsenosides. As Choi et al, [9] proved, Rb2 and Rc increased GAD activities in a dose-dependent manner in vitro in response to increasing concentrations of Rc Among the GABA shunt enzymes only the GAD activities were increased after total ginsenosides treatment in vivo [9]. Upon the previous results that ginseng has a suppressive or anticonvulsive activity Jin-Kuy Park and coauthor [23] studied the effects of ginsenosides in the immature rats. 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/rat b.w.) using 10 day-old male. Kainic acid (KA), an analogue of the naturally occurring excitatory amino acid neurotransmitter glutamate. Diol ginsenosides such as Rb1,

Rb2, Rc and Rd generally reduced the seizure activities while triol ginsenosides such as ginsenoside-Rg1 and Re rather increased stereotypic "paddling-like" movement although decreasing the more severe seizures like a loss of balance, and tonic clonus or final upset caused by an apparent lack of consciousness. It was demonstrated also 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 [23].

The last research of reduction of electrically evoked neural activity by ginseng saponins in rat hippocampal slices demonstrated that ginsenosides did not induce chloride current nor modified GABA-induced current. Taking into account that suppressive effect ginsenoside on EPSPs was still observed in the presence of the GABAA receptors antagonist (bicuculine) it could suggest that suppressive effect is not attribute to regulation of $GABA_A$ receptor activation [18].

Acetilcholinergic system could participate in the ginsenosides effects. The most important finding in our laboratory was that the i.c.v. administration of galanin led to a decrease in the spike-wave activity of WAG/Rij rats and this action is mediated through the galanin receptors. M15, antagonist of GalR1 and GalR2 receptors, blocked these effects. As far as M15 show some preference for GalR2 it could be concluded that this type of GalR as far as GalR1 is involved in the onset of SWDs [3].

## Conclusions

All the models of epilepsy that we have studied had shown the antiepileptic effect of ginsenosides. The development of optimal ratio of different ginsenosides should consummate in the new antiepileptic drug. The chemical structures of ginseng extracts have some principal differences from well-known antiepileptic drugs but plant pharmacology gives us unique possibility to developed new class of antiepileptic drugs and to improvement its biological activity. Especially we were encouraged by the significant advances in the field of ginseng research [1, 16-20, 24, 25] that have been achieved at the fundamental scientific level and in clinical practice.

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