The Inhibition of Epileptogenesis During Status Epilepticus by Ginsenosides of Korean Red Ginseng and Ginseng Cell Culture (Dan25)


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Abstract: Pharmacology of Korean Red ginseng gives us unique possibility to develop new class of antiepileptic drugs today and to improve one’s biological activity. The chemical structures of ginsenosides (GS) have some principal differences from well-known antiepileptic new generation drugs. The antiepileptic effect of GS was also demonstrated in all models of epilepsy in rats (young and adult), which have studied, in all models of epilepsy including status epilepticus (SE), induced by lithium - pilocarpine. In our experiments in rats new evidences on protective effects were exerted as a result of premedication by GS. Pre-treatment of several GS could induce decrease of the seizures severity and brain structural damage (by MRI), neuronal degeneration in hippocampus. Wave nature of severity of motor seizures during convulsive SE was observed during lithium-pilocarpine model of SE in rats (the first increase of seizures was 30 min after the beginning of SE and the second - 90 min after. The efficacy of treatment on SE by ginsenoside as expected was observed after no less 3 weeks by daily GS i.p. administration. It is blocked SE or significantly decrease the severity of seizures during SE. The implication of presented data is that combination of ginsenosides from Korean Red ginseng and ginseng cell culture Dan25 that could be applied for prevention of epileptical status development. However, a development of optimal ratio of different ginsenosides (Rb1, Rc, Rb2, Rd, Rg1, Re, Rf) should consummate in the new antiepileptic drug development.

Keywords: ginsenosides: Rb1, Rc, Rb2, Rd, Rg1, Re, Rf, epilepsy, status epilepticus, severity of seizures, MRI, rat brain imaging

INTRODUCTION

The results of cooperative studies held by Lomonosov Moscow State University (Russia) – KT&G cooperation suggest that the antiepileptic properties of ginseng and/or various ginsenosides (GS) of Korean Red ginseng, which administrated intranasally in a systematic way, be different with the new generation anti-epileptic drug in the relief of seizures or several models of seizures or epileptic fits. Simultaneous and positive effects on spatial memory coped with the worsened cognitive function by the seizures that accompanied with experimental amnesia were also demonstrated. It has been uncovered that GS could have binding with GABA receptors, thus start creation of the inhibitory and antiepileptic inside system in brain. There was a possibility of applying this medication in this treatment of diseases, which can negatively affects on memory and cognitive function. GS has a protective property on liver when is used as combination form with antiepileptic drugs, and it is also effective in the period of reconvulsive after neurosurgical treatment of epilepsy or a neuronal oncology. It has been correlated with neuroprotective activity of Rb1 after hypoxic injury in young rats, calcium independent CamKII activity may be involved by GS in the recovery of neuronal cells after hypoxic damage in seizures.

Epilepsy is the result of highly complex structural, bio-
chemical, electrophysiological changes these occur at various level not only within the brain but also in the entire body. We have experience of ginsenosodes application for purpose to inhibit the seizures with a difference in the etiology, which were presented at The 8th International Symposium on Ginseng in Seoul in 2002 (11) and at The 1st Beijing Epilepsy Forum in 2004.

New study on ginsenosides in decreasing brain excitability were applied using the model of status epilepticus (SE) (22). Status epilepticus is the most dangerous increasing of pathological activity of brain leading to loss of the consciousness and failure of all regulations of vital functions on the border of survival. The International Classification of Epileptic Seizures defines SE as a seizures lasting for more than 30 min or intermittent seizures lasting more 30 min from which the patients does not regain consciousness. Any type of seizures may become prolonged, it may develop into SE. W.A. Hauser reported in 1990, that SE occur in 50 000 – 60 000 individuals in the US every year (23). D. Lowenstein and B. Allredge reported in 1998 for Europe - 150 000 individuals every year (unfortunately 55 000 from them died) (24). The understanding of the mechanisms and complication of SE at the molecular level, which should eventually lead to improve therapy, and treatment strategies today have a great sense of urgency because of the realization that neuronal apoptosis and necrosis can be triggered very quickly (25).

In animals SE can be produced by the administration of pilocarpine. In this model, the preadministration of lithium substantially reduced the dosage of pilocarpine to induce seizures (26, 27). Lithium is known to synergies the action of cholinomimetics in the CNS such that pilocarpine, but lithium does not synergies the action of cholinomimetics in the periphery (blood pressure and ileum contraction measured) as that seen in the CNS (28).

Following the termination of SE many subjects show, after a delay of several weeks, developed spontaneous recurrent seizures that resemble human temporal lobe epilepsy. After investigation by W.A. Turski (26) and E.A. Cavalerio (27, 29) this model of SE is reminiscent of that human temporal lobe epilepsies which often begins with prolonged SE in infancy and develops with recurrent seizures in later life. Lithium-pilocarpine model is one of model of partial limbic seizures that progress to secondary generalized SE. The epileptogenic effect of cholinergic agents depends on the facilitation of burst discharges in hippocampal pyramidal neurons by means a block of the K⁺ transmembrane current. Super-activation of neurons of hippocampus during pilocarpine-induced SE leads to cell death. Seizures–related excitotoxicity involves glutamate receptors and Ca²⁺ influx (30). The EEG is helpful in further dividing SE into those that are generalized from onset, or have a partial onset. The investigation of thresholds and time of SE duration in Li-pilocarpine model is connected not only with blockade of muscarinic (M₁ - M₅) cholinergic receptors, as noted Berkely et al (31) but also with the basic problem of GABAₐ receptors desensitization (32).

The main aim of study was researching the possibility of treatment by GS during or before SE.

**MATERIALS AND METHODS**

Control registration of basic EEG was done during interictal periods for evaluation of chronic epileptogenesis. Motor seizures were evaluated with presently accepted scale of motor convulsions in rats. It was evaluate the sets of correlation between neocortex EEG patterns of brain and behavioral manifestation of fits (video record).

<table>
<thead>
<tr>
<th>Table 1. The scores of behavior and motor seizures during lithium - pilocarpine status epilepticus</th>
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<tbody>
<tr>
<td>5 Very severe tonic-clonic seizures with falling</td>
</tr>
<tr>
<td>4, 5 Tonic-clonic seizures</td>
</tr>
<tr>
<td>4 “Kangaroo pose” tonic seizures body muscle</td>
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<tr>
<td>3, 5 Fast jerk (several s. duration), bucking on the hind limb</td>
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<tr>
<td>2, 5 Minimal seizures, clonus head muscles</td>
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<tr>
<td>3 Clonus head and limb muscles, jerk of body, jumping</td>
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<tr>
<td>2 Atipical minimal seizures, body jerk</td>
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<tr>
<td>1 Single mioclonic spasm, years jerk, head sake</td>
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<tr>
<td>0, 5 Grooming, hiding behavior (abnormal behavior)</td>
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<tr>
<td>0 Normal behavior, no change</td>
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The characteristic of lithium-pilocarpine-induced SE

The subjects were 3-4 months old (n=41) and 6-8 months old (n=30) Wistar rats. In accordance with the protocol (Fig. 1) lithium chloride was prepared as a stock solution in saline (3 mEq/kg - 127 mg/ kg body weight) 24 h before injection of pilocarpine. In this protocol the concentration LiCl in the blood should be 10 h after injection – 0.69±0.03 mEq/l, 24 h after injection – – 0.16±0.01 mEq/l. Pilocarpine hydrochloride, was administered at in 2 ml of saline. Following injection of pilocarpine, the rats were observed continuously for behavioral severity of seizures using modified five-stage classification, of severity of seizures (Table 1).

The behavior motor fits were observed in special boxes (25×30×25cm). Latency of motor fits and severity of the seizures were measured. Animals were observed for up to 2-3h, followed by paraldehyde («Acrus») administration in concentration 0.6 ml/kg (0.1 ml paraldehyde and 0.9 ml of distilled water) to stop SE. The survival of animals after SE was the main problem of developing of chronicle epilepsy. Immediately blocking of SE by diazepam is required after clonic-tonic stage. These animals could be saved during long time after several SE and used them for the further analysis of brain damage by MRI imaging. Mortality was determined 24 h after experiments.

Magnetic resonance imaging was performed under the Nembutal narcosis on BRUCKER Biospec 70/30 (Germany) Bore MRI system. Ten coronal or frontal images with a slice thickness of 1.5-2.0 mm were acquired with a multislice gradient echo sequence: 40 mm field of view (FOV). High resolution gradient echo images were acquired with an echo time (TE) of 6.9 ms, a repetition time (TR) of 500 ms an acquisition matrix 200×400.

GS were received from KT&G (Daejon, Korea). The callus cell culture Dan25 from ginseng root was used. The ethanol and butanol extracts and lyophilic substances of ginsenosides were conducted in Joint-stock association “Biokhimnash”, Moscow. The content of extracts was evaluated (Rb1, Rb2, Rc, Rd, Rg1+Re, Rf) ginsenosides - 7,552 mg/g - biomass of the extract. GS were dissolved in physiological solution (24 mg/ml), filtrated and injected intraperitoneal in concentration 18 mg/ kg. The GS injected at same time.

Stereotaxic method was used for microelectrodes insertion into the cortex and hippocampus under the deep Nembutal narcosis (47 mg/kg) and local analgesic (Novocain 1%, «CEV A»). Rats received 5 ml glucose and 0.3 ml Gammavit after operation. EEG record was performed 7 days late.

All experimental protocols used were in complicate with standard and were approved by the European and Moscow University Animal Care and Use Committee.

The latency to seizures onset, duration of status, seizures score were averaged across animals in the same treated group. Comparison between groups were done with an analysis of variance (ANOVA/MANOVA), Criterion “U” (Mann-Uinty) to takes account of BIOSTATIC. A statistical difference was determined by a value of p<0.05.

RESULTS

The characteristics of lithium- pilocarpine-induced SE

Experimental status epilepticus was induced in adult rats. Before the SE the reactivity on the pilocarpine also registered. It was connected with muscarinic receptors activation. After injection of pilocarpine strong salivation, defecation,Urination (infrequent ejaculation) were observed 7-15 min after pilocarpine injection. Sudden quick running and head jerk were not compulsory seizures precursor. This model was also char-
acterized bilateral hind limb struggling, grooming. It was highly repetitive grooming motion with its hind limb without making contact with its body. The first 15 min following pilocarpine administration the animal can exhibited a brief pause in motor activity. In our experiments the aggressiveness of rats after SE was increased if the SE did not develop the rats be restored after 3 h.

Normally spike and spike-wave discharges in cortex EEG were not observed prior to pilocarpine injections it was correlated with motor seizures only. Wave pattern progressively increasing in amplitude and EEG correlated of seizures stage (Fig. 2). SE was stoped by paraldehyde.

The relative latent period was estimated as the time between the injection date and the first observed motor seizure (direct or in any experiments – video monitoring). Latency of SE in rats developing in average was 35 min (have ranged 5-90 min). As usually it begins from 4th score of seizures (extensor reflexes), The duration of which was from several seconds to minutes. The developing of SE include mouth and facial automatisms, head nodding, forelimb clonus, rearing, and rearing with falling. After the jumping the seizures pass on 4th score of fits. Our data in the agreement with results of study SE in rats\textsuperscript{32, 36, 37} and in Guinea pigs\textsuperscript{38}.

For any animals smooth developing of SE from 1st to 4th score of seizures was observed. Seizures of 4th score could be recurred.

Wave nature of severity of motor seizures during convulsive SE was observed during lithium-pilocarpine model of SE in rats (the first increasing of seizures was 30 min after the beginning of SE and the second - 90 min after. Fig. 3.

Some rats demonstrated during SE the limbic epistatus, which characterized by jog reaction - “wet dog shake”.

The effects of GS on SE were studied on two experimental protocols: with bolus administration of GS (n=36): (series 1-3) and with chronically (everyday) administration of GS (n=25) (series 4-6).

**EXPERIMENTAL PROTOCOL 1.**

**The effects of bolus GS injection**

Series 1(n=12) – bolus injection of GS - 30 min before pilocarpine;

Series 2 (n=9)- – bolus injection of GS - 30 min after pilocarpine SE has begun;

Series 3 (n=15 ) – control saline administration.

The latent period of SE was in control series 38.3+6.9 min in the case of GS injection before pilocarpine – 28.9+2.2 min, but if the GS injected after the SE begins –

**Fig. 2.** EEG activity during status epilepticus in rat. 1- EEG recorded in frontal cortex, 2,3 –the first predictor of SE inhibition was the increasing the interval between spike-wave discharges.

**Fig. 3.** The two-wave character of pilocarpine SE in rat (single animal from control group).
38.8±4.1 min. The number and duration of seizures 4 and 5 score were also measured (together 4+5 score) and separately only 5th score. Fig. 4. The difference between group of rats in all series were not significance. More demonstrative was the number of 5th score of seizures: in control series 3,3±0,9, in experiments with GS infection before pilocarpine 1,7±0,2, but for group with GS injection after the beginning of SE – 2.3±0.5 (Fig. 4-C). In these experiments the decreasing of “wds” was observed (Fig. 5).

These results permit to make resume that bolus GS administration during SE absolutely non effective, but pretreatment by GS just before pilocarpine injections could inhibit the SE developing. Probably the amount of GS should be increase in bolus injection.

It turned out, that the number of rats, which demonstrated SE is the considerable and manifesting index. If before pilocarpine was injected saline only the 75% of rats developing SE. In group of rats with GS injection 30 min before pilocarpine SE developed in the 58% of cases.

We could suppose that in the inhibition of SE could participate several mechanism of GS action. It could be prolongation of IPSP in the hippocampal cells, inhibition of recapture of transmitters and NMDA increasing of intrac-
Cellular calcium. All these mechanisms decrease seizure excitability of brain and providing the antiepileptic brain system of self defense. It means that the time of GS action and supporting into the blood and brain of GS concentration will be more effective.

**EXPERIMENTAL PROTOCOL 2.**

**The chronically GS injection.**

In a different way performed the chronic administration of GS. In Series 4 (n=5): GS administered i.p. for 6 days (on the 5th day, injected together with LiCl and then on 6th
day injected 1 h before pilocarpine) The experiment repeated three times and consumptive GS use was 3 weeks.

New group rats was tested on the pilocarpine-induced SE development. After that the rats was divided into two equal subgroup series 5 and series 6. In Series 5 (n=5): rats were received daily GS for 4 weeks (extract 18 mg/kg), and then experienced 3 lithium-pilocarpine test. It should be written as “test”, because SE was not developed in the majority of rats in GS-group. Series 6 was control rats, that received saline only and tested 3 times of lithium-pilocarpine.

It was suggested that daily GS injections as pretreatment will be more effective in the SE inhibition. These experiments were uncovered by the individual differences. As presented at Fig. 6 and Fig. 7, actually the severity of motor seizures during SE was actually decreased. The high 5th score of seizures not observed in all male adult rats.

As presented in the Fig. 7 one week GS treatment induced SE without 5 score of seizures, but two wave of motor fits presented. But 3 weeks of GS treatment induced inhibition of SE, but the status epilepticus development was accompanied significantly decreasing the first wave of fits (Fig. 8).

Single-factor analysis of variance shows a significant decreasing the severity of seizures during SE, if it developing after 3 weeks GS treatment.

The inhibition of epileptogenesis connected with muscarinic receptors binding (central effect of pilocarpine) was demonstrated after chronic GS administration (Fig. 9).

The effect of GS could be evaluated as relieved on the seizures development and the duration. The first wave of seizures 4-5 score was completely excluded, but the second wave was never excluded more 3rd score of seizures. The decreasing severity from 4-5 score to 3rd score demonstrated significant inhibition of epileptogenesis.

Motor seizures during expand in the time. This process of SE developing could be evaluated by measuring of stage of severity of motor seizures. The comparison of frequency of occurrence different stage in control group (saline injection) of rats and experimental group rats, treated GS shows that only in experimental group the significant increase number of seizures 1-2.5 score, and the number of seizures 3-3.5 score decreased. (Fig. 10). GS decrease severity of response of brain on the pilocarpine action.

Fig. 7. Dinamic of epileptogenesis during Li-pilocarpine status epilepticus in rat after two (above) and three weeks (below) of GS treatment.
Fig. 8. Longtime effect of GS treatment during 4 weeks, SE pilocarpine test was repeated after 4 weeks of treatment of GS.

Fig. 9. Summarized average data of SE developing, Significant decreasing the severity of SE in experiments with chronic GS injections. Key top-down: 1-control, 2-injection of GS before pilocarpine, 3-injection of GS after the SE beginning, 4-chronic injection of GS 3 weeks.
The closing series of experiment was performed on Wistar rats, 6-8 months old (n=15 saline; n=15 GS injection). The SE before GS treatment was compared with after 4 weeks of GS injections. The result demonstrated on Figs. 9 The severity of seizures in control rats did not change. The mortality was high in control group. Single-factor analysis of variance shows the significant difference between SE test before GS and after GS treatment only, but not for control group with saline injections. It was noted before that SE developed in control group (n=24) in 75% of cases, but in experiments with chronic GS injection SE (n=14) developed in 43% of cases (Fig. 11).

It is possible to suggest that chronic daily treatment of GS induce significant inhibition or significant decrease in the severity of SE.

However, it should be correctly noted that the most of rats treated by GS were completely not “flow into” SE.

MRI imaging rat brain and diagnostic of long-term alterations of brain tissue following status epilepticus.

A number of electrophysiological and neuroimaging procedures are used for the presurgical localization of the epileptogenic focus. Together with clinical data, noninvasive imaging procedure such as MRI, PET, SPECT etc., are used to provide information on the focus that can be further investigated using invasive EEG recordings. MRI are based on the same physical principle, as indicated by the first two letter of their acronyms: they measure the resonance frequencies (of protons) after stimulation by high-frequency radio pulses in high, steady magnetic fields. Our idea was to determine what is brain
alterations in MRI after several cases of SE. MRI was performed in the agreement of the recommendation of N. van Camp et al.\textsuperscript{34}.

All MRI slices of each animal (5 – control, 5 with GS) were examined, but for final analysis only slices were considered that include relevant brain structures. The imaging of normal rats and treated GS rats was studied. As shown on the Figs. 12-16, there is a focal damage in the hippocampal structures, piriform lobe, cortex and different pathology of brain ventriculus. A priory conclusion is that long-term abnormalities in rats one months after several SE could be bring to light by MRI. It is important data that this over patching registered one months after 3 or 4 SE. All rats treated GS (it means the decreasing of severity of SE) not observed brain abnormality, as compare with control rats.

The temporal evolution of the lesions in the lithium-pilocarpine model of epilepsy in the rat with MRI to determine the progressive morphological change occurring before he appearance of chronic epilepsy was studied by Catherie Roch and co-authors\textsuperscript{40}. The authors studied MRI 2 h, 6 h and 24 h after SE. It was demonstrated also that in the hippocampus, the correlations between histopa-
thology and T2-weighted signal underscored the progressive constitution of atrophy and sclerosis, starting 2 days after SE. The results of repeated SE did not investigate.

Our data permit to make the conclusions about wave nature of severity of motor seizures in rats during motor convulsive SE, induced by lithium-pilocarpine (the first increasing of seizures was 30 min after the beginning of SE and the second - 90 min after. The efficacy of treatment SE by GS as expected observed after no less 3 weeks (daily GS i.p. administration). It is blocked SE or significantly decrease the severity of seizures during SE. The implication of presented data is that combination of GS from Red Korean ginseng and ginseng cell culture Dan25 could be applicated for prevention of epileptical status development.

DISCUSSION

Ginseng has inexhaustible potential, as effective remedy to preserve of health of Nations in the World. It is supported the most new effort with Korean Red ginseng using the modern molecular and genetic methods and new neurobiological ideas. Saying figuratively “ginseng one kept the secretions of all pharmacological company have taken together in East and West”.

There are many targets in our suffering body, where its compound have therapeutic action. This is the problem of biological sciences to cover and to know these targets in the harmony of all absolute rich ginseng substances. So, the question is: what we should recommend for any decease treatment: ginseng? ginseng’s extracts? ginsenosides complex? single ginsenoside as high specific biochemistry regulator of specific biological function in organisms. Pharmacological effects attributed to GS have been shown in the central nervous system, the cardiovascular system, the endocrine system, and the immune system. They are thought to have antineoplastic, antistress, and antioxidant properties. Now we stored knowledge how the GS act at the levels of organism, organs, tissues, cell, membrane, channels and genes.

There are no the insuperable contradictions today between using ginseng of official medicine and complementary and alternative medicines (CAMs), including herbal medicines. It has a long history of use across several cultures to treat a variety of ailments, including memory loss, if it connected presumably with cholinergic system of brain. Much really scientific research has been published on the pro-amnestic effects of ginseng in animal studies, but evidence for such effects in humans unfortunately is lacking. As M.Spinella wrote not long ago in 2001 42% of Americans surveyed have recently used at least one such form of CAM therapy. A large proportion of CAM consumers (40%) do not dis-

Fig. 15. (f) (sequential) MRI imaging the same slice of rat brain. Rat received GS 4 weeks and 3 SE. (rat eOg2r4_sc5s15ec1_GS).

Fig. 16. (g, h) The frontal slices, coronal scan of rat brain. Rat expired 3 SE and received GS during 4 week (different echo and MRI regime) (slices: eOg2r4s17ec1_GS and eOg2r4s17ec2_GS).
close their use of CAMs to their physicians, and it was estimated that 15 million American adults took prescription medications concurrently with herbal medications in 1997. It is very important that 24% of people with epilepsy in one tertiary care epilepsy clinic reported using CAMs.41

The maximal questions should emerged concerning the central effects of GS. Where are documentary scope using GS in neurology?

The main contradiction arises every time, when the questions of application of ginseng in neurology discussed. On thing is the anti-epileptical efficacy of different combination of ginsenosides, another thing is the note such as: …most of the ginseng available in the American market is made from the root of American ginseng suggests, that people with epilepsy, especially those with history of status epilepticus, should not take ginseng45.

Our presented new data are accordance with the results demonstrated these authors. Enriched Rb1 extracts really have anti-convulsant effect. But we cannot agree with assumption that “some ginsenosides are anticonvulsant, but that other component of the whole extract from roots or leaves/stem are proconvulsant or alternatively contain components that neutralize the anticonvulsant affect of the active components “ [45, p. 21.] However there are not direct evidence which kind of substances from ginseng extract (e.g., in form of tea powder) have proconvulsant effect. Without doubt the problem of efficacy of purified GS in clinic of epilepsy will be required further testing.

Park J-K.46, 47 and Lee J-H.48 convincing demonstrated on the kainite model of SE that GS (e.g. - Rd) have protective effect on the neurotoxic response of brain. Pretreatment with whole ginseng extract has been shown also to reduce the neuronal loss.48, 49, but we not need to separate the anticonvulsant effect and neuroprotective effect, because reduction of convulsions leads to minimization of neurons death. We should underline that pretreatment with GS (obligatory include Rb1) have curative action in rats manifesting in the abolish or significant decrease in experimentally induced SE lithium-pilocarpine model.

Lian X-Y et al.45 demonstrated using three models of seizures (PTZ induced seizures, pilocarpine-induced SE and kainite-induced SE) that fixed combination of GS has anticonvulsant properties. Applied partial purified extract content Rb1 (24.8%), Rb3 (46.4%) and Rd (13.1%) It is agree with our earlier publication about ginsenoside Rb1 has anticonvulsant effect45.

Using lithium-pilocarpine model of SE has neurochemistry specificity: 1) its is result of cholinergic muscarinic receptor activation, 2) deep damage of brain could be observed after SE. Exactly that character of lithium-pilocarpine-induced SE de fide that SE is fruitful animal model of temporal lobe epilepsy (TLE) in human. Many TLE patients develop pharmacoresistance: seizures can no more be controlled by antiepileptic drug. Animal models of TLE appear particularly helpful to study molecular mechanisms of hyperexcitability and pharmacoresistance and to new generation of antiepileptical drugs, including GS.

New experimental methods of neurobiology give us possibility to understand several mechanisms of GS action. The ginsenosides be drawn into regulation of synaptic transmission have beneficial effects on protecting neuronal or non-neuronal cells from degeneration. For example, GS modulate neurotransmitter release by inhibiting the uptake of GABA, glutamate, and biogenic amines in the presynaptic terminal50. They also act on the postsynaptic dopamine receptors51 and GABA receptors17,52,53, or Ca2+ channels54, and modulate the efficacy of synaptic transmission55. It was reported that in vivo treatment of ginsenoside Rb1 protected hippocampal CA1 pyramidal neurons from lethal ischemic damage56,57 and improved learning and memory probably by facilitating also cholinergic function58. It is possible to assume that one new mechanism of antiepileptic action of Rb1 (and probably, other ginsenosides) in pilcarpine-induced seizures consist in the modulation of extracellular signal-regulated kinase 1/2 signaling cascade31.

The role of NMDA receptors ascertained in the mechanisms of epilepsy, stroke and other neurodegenerative deceases. NMDA receptor-mediated pilocarpine-induced seizures, that demonstrated Smolders et al.59 in freely moving rats by microdialysis. With regard these data we must draw for explanation GS effects by new results of Kim S. et al.60, 61 discovered, that GS could inhibit NMDA receptor-mediated epileptic discharges in cultured hippocampal neurons. This is clarify itself both effects – anti-seizures and protective neurons death. Ginsenoside Rg2 was shown to enhance neurogenesis of dentate granule cells, which may contribute to functional recovery of hippocampus from ischemia62. It was further shown that Rb1 can protect spinal cord neurons from glutamate toxicity and induce neurite outgrowth63, which might involve protein synthesis of neurotrophic factors at the gene expression levels64. All these studies strongly indicate that GS may function as a positive modulator for neurons undergoing degeneration after injury. Because problem of SE connected with severe hypoxia, it is impor-
tart to draw date, recently presented by Park J-K.,\textsuperscript{20} It was shown that that calcium independent CaMKII activity may be involved in the process of ginsenoside Rb\(_1\)-mediated recovery of neuronal cells after hypoxic damage. The pretreatment with Rb\(_1\) prior to hypoxic stimulation reduced animal death to 12\%, and also significantly reduced the recovery time from hypoxia-related. Rb\(_1\) also significantly reduced levels of lactate dehydrogenase release from primary hippocampal neurons, indicating increased neuronal survival by Rb\(_1\).\textsuperscript{20} The normalization of brain neuronal networks during SE by GS promote the direct effects of GS on the base mechanisms of neurons excitability or inhibition. In such a way, GS could modify electrical evoked neural activity in rat hippocampal slices.\textsuperscript{65} Lee J-H\textsuperscript{66} recently shows that ginsenoside Rg3, could mediated brain Na\(^+\) current inhibition. The short-term changes (Kv4.2potassium channels) and the long-term changes (loss of selective type of interneurons, excitatory circuits by mossy fiber sprouting) that promotion the epileptic state and recurrent seizures in limbic structures by Rb\(_1\).\textsuperscript{20} The normalization of brain neuronal networks during SE by GS promote the direct effects of GS on the base mechanisms of neurons excitability or inhibition. In such a way, GS could modify electrical evoked neural activity in rat hippocampal slices.\textsuperscript{65} Lee J-H\textsuperscript{66} recently shows that ginsenoside Rg3, could mediated brain Na\(^+\) current inhibition. The short-term changes (Kv4.2potassium channels) and the long-term changes (loss of selective type of interneurons, excitatory circuits by mossy fiber sprouting) that promotion the epileptic state and recurrent seizures in limbic structure are connected with cholinergic mechanisms. GS could include in the processes of normalization cholinergic transmission and long-term effects of apoptosis\textsuperscript{67} and neuron survival\textsuperscript{68}.

It is follow in the conclusion to remind about complicity of GS in stress reactivity and influence on the pituitary–adrenocortical system. Long-term premedication with GS, especially Rg\(_1\) (a functional ligand of glucocorticoid receptor), could involved endocrinological defense mechanisms\textsuperscript{69,70} during status epilepsy.

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