Antiepileptical Properties Of Ginsenosides From Korean Red Ginseng And Ginseng Cell Culture (Dan25).

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The molecular modification of antiepileptic drugs and direct synthesis of new drugs with the predetermined antiepileptic properties are perspective. New neurochemical attacking to solve the problem including prevention and inhibition of seizures seems to be related to ginsenosides and ginseng polypeptides. The main study based on the severity of febrile convulsions of rat pups has been done from the earlier investigations of antiepileptical action of ginsenosides between KGTRI and MSU (Chepurnov, Park et al., 1995) with different kinds of experimental models of epilepsy. From the cultured cell line DAN25 of ginseng root, the extracts of ginsenosides made in "BIOKHIMMASH" were studied by the project of preclinical anticonvulsant screening (Stables, Kupferberg, 1997). The inhibition of severity of convulsions, decrease of seizures threshold, decrease of audiogenic seizures in rats of different strains and normalization of cerebral blood flow (measured by hydrogen test) were demonstrated in rats after i.c.v., intraperitoneally and orally administration, respectively. The antiepileptical

effects by the combination of compounds from ginseng were compared with the influence of Rg1, Rb1, Rc and with the well known antiepileptical drugs such as carbamazepine, valproic acid. The base for the research is obtained by using the WAG/Rij strain (Luijtelaar, Coenen, Kuznetcova), an excellent genetic model for human generalized absence epilepsy. The improving action of gensinosides was effectively demonstrated on the model of electrical kindling of amygdala of WAG/Rij rats with genetically determined absences, and the influences of ginsenosides on the slow wave discharges have also been being investigated. The different characteristics of a kindling process exerted in the sex-different region of the amygdala and demonstrated that the level of sex steroids and content of neurosteroids in amygdaloid tissue can modify the development of seizures.

The chemical structures of ginsenosides not only have some principal differences from well-known antiepileptical drugs but the plant pharmacology gives us unique possibility to develop new class of antiepileptic drugs and to improve its biological activity.

Introduction

Phytotherapy in neurological diseases and in particular in epilepsy [9, 13] are being widely used, although the study of nature of this disease and understanding the role of excited aminoacid and GABA allowed to develop new pharmacological drugs that control epilepsy in a very good way. However over 20% of patients are resistant to treatment in European way. This can be very solid base for further implementation of traditional Eastern medicine in which ginseng has a leading role [4].

In 1994/1995 in cooperation with Jin Kyu-Park (KGTRI) we have collected proves that components of ginseng extracts and some definite gensinosides (Rb1, Rg1, Rc) possesses antiepileptic properties. This was shown in experimental models of epilepsy in animals. For the first time using the model of child febrile

convulsions (heating-induced seizures in rats pups) the decreasing the severity or complete inhibition of fits by ginsenosides [2-4]. Further fundamental research has proven the effects of ginseng that explains it (antioxidant, cytoprotection, neuroprotection, calcium channel inhibition and inhibition effect to reuptake of neuromediators, modulating to specific binding of GABA a and GABA b receptors).

The aim of this study was the research of antiepileptic activity of gensinosides in different kind of experimental models of epilepsy.

Methods and Materials

The callus cell culture DAN25 from ginseng root was used [7]. The ethanol and buthanol extracts and theirs lyophilisation of gensinosides was conducted in Joint-stock association (Biochimmash). The content of exstracts was evaluated (Rg1+Re, Rf, Rb1, Rc, Rb, Rd,).

The physiological effects were studied on the preclinical anticonvulsant-screening project (Stables, Kupferberg, 1997). The inhibition of severity of convulsions induced by chemoconvulsants, decreasing of seizures threshold, decreasing of audiogenic seizures in rats of different strains, normalization of cerebral blood flow (measured by hydrogen test) and were demonstrated in rats after introcerebroventricularly (i.c.v.) intraperitoneally and orally administration.

The seizures severity were evaluated by using five-score scale described by Mares P., H.Kubova [12]: 0-no changes in behaviour; 0.5-atypical behaviour (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 (generalised 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 hindlimbs) progressed to the clonus of all four limbs.

The antiepileptic effects of combinations of compounds from ginseng were compared with the influence of chemically poor Rg1, Rb1, Rc, and well know antiepileptic drugs - carbamazepine, valproic acid.

The base for the research was the discovery G.Luijtelaar and A.Coenen [5, 6], that the WAG/Rij strain is an excellent genetic model for human generalized absence epilepsy. The experiment was 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. Numbe r, the mean duration and total duration of spike-wave discharges were measured. The electrical activity of WAG/Rij rats brain with genetically determined absences was researched and the change of spike discharge wave (SWD) served as a prove of effectiveness of gensinoside.

Results

In rats with audiogenic epilepsy (KM line) the effectiveness of introperitonial administration of gensinosides was shown (Fig.1). The latency for the 3rd and 4th stages of convulsive response significantly increased, and that means the seizure threshhold increased. 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 gensinosides (Fig. 1, -1, 2).

Penthelenetetrasol was used as chemoconvulsant. This model also proved the antiepileptical effect of gensinosides. The intraperitonial administration of gensinosides (5-7 mg/kg) induced the increasing of latency of clonic-tonic fase of seizures (Fig.2 A, 2). The duration of seizure response significantly decreased (fig.2, -B). Thus, we can say that gensinosides are effective even when hard experimental model like PTZ (which induces seizures potentiated by GABA agonist) is used.

In a separate series of experiments it was shown that the effect of gensinosides as adaptogens has individual characteristics. Based on the method that was

developed by Gorkavy at al, 1978 we have researched the effect of gensinosides on the condition of white blood. Gorkavy at al have shown that the percentage of lymphocytes from leykocytes is an indicator of adaptive reaction of organism.
We have shown that gensinosides administration increases the number of lymphocytes. And if the epileptical seizures in these rats are induced by PTZ administration the severity of epileptical response correlates with the functional condition of white blood.

After gensinosides 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, -A, 2). In the same group of rats that had developed epilepsy the latent period of tonic-clonic phase was three times higher (Fig.3, -B, 2). The same results we got when we used the root extract (Eleutherococcus senticosus)(Fig3, -A, B, 3). These results show the correlation between nonspecific adaptation reaction of organism and seizures readiness

The improving, effective action of gensinosides effective demonstrated on the model of electrical kindling of amygdala of WAG/Rij rats with genetically determined absences, the influences of ginsenosides on the slow wave discharges investigated now. On the 4th Figure the results of continues registration of electrical activity of neocortex in rats is presented. The column shows the absence period, the size of which represents the duration of absence (SWD). The control administration of artificial cerebrospinal fluids didnt change the periodic character of absence (Fig.4, -A). After i.c.v. administration of gensinosides 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 .

The conclusion

All the models of epilepsy that we have studied had shown the antiepileptical

effect of gensinosides. The development of optimal ratio of different gensinosides should consummate in the new antiepileptical drug. The chemical structures of ginseng extracts have some principal differences from well-known antiepileptic drugs but plant pharmacology gives us unic possibility to develope new class of antiepileptic drugs and to improvement its biological activity. Especially we are incouraged by the significant advances in the field of ginseng reaserch [1, 10, 11] that have been achieved at the fundamental scientific level and in clinical practice.

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