UDC 616.008+615.9:547.002

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PROEPILEPTOGENIC EFFECTS OF SILVER NANOPARTICLES ON PENTYLENETETRAZOL-INDUCED KINDLING

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ПРОЕПІЛЕПТОГЕННИЙ ЕФЕКТ НАНОЧАСТИНОК СРІБЛА НА МОДЕЛІ ПЕНТИЛЕНЕТЕТРАЗОЛ-ІНДУКОВАНОГО КІНДЛІНГУ
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На моделі пентиленететразол (ПТЗ)-індукованого кіндлінгу у щурів лінії Вістар показано зниження середньоефективної дози ПТЗ, що викликає клонічні судоми у 50 % щурів під впливом системного застосування наночастинок срібла. Даний ефект був більш вираженим у віддаленому періоді кіндлінгу й супроводжувався посиленням електрографічних епілептиформних проявів. За умов хронічного експерименту проводили досліди із визначення впливу наночастинок срібла на вираженість хронічної судомної активності. Автори висловлюють думку, що підсилення інтенсивності судомної активності відбувається завдяки придатності наночастинок срібла.
Ключові слова: пентиленететразол, кіндлінг, наночастинки срібла, епілептична активність.

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On the model of pentylenetetrazol (PTZ)-induced kindling in Wistar rats the net reduction of ED_{50} dosage of PTZ, which was able to induce clonic seizures in 50% of experimental animals caused by silver nanoparticles was established. This effect was more pronounced in postponed period of kindling and was followed by intensification of electrographic epileptiform manifestations. Silver argentum nanoparticles influence on chronic seizure activity was determined in conditions of chronic experiment. Authors concluded that argentum nanoparticles are responsible for seizure activity enhancening.
Key words: pentylenetetrazol, kindling, silver nanoparticles, epileptic activity.

Introduction

Nanotechnologies possessed prominent role in the treatment of some diseases affecting brain tissue [4; 5]. Namely metal nanoparticles proved to be effective in course of treatment of brain tumors [4], while neurotropic agents being encapsulated in polymeric nanoparticles show good bioavailability and are able effectively overcome blood-brain barrier [7]. It was shown that water insoluble antiepileptic drugs in a nanoform displayed excellent pharmacokinetics after systemic administration [7].

Phenytoin-contained liposomes demonstrated high level of antiseizure activity on a model of seizures induced in rats with cAMP/EDTA, and nanoparticles of blocker of NMDA receptor MRZ 2/576 exceeded antiseizure activity of this compound delivered in free form by 10 times [7]. It was shown that clonazepam being incorporated into solid lipid nanoparticles demonstrated better penetration through BBB and improved ability to prevent PTZ-induced generalized seizures [5]. But the penetration worsened for clonazepam in mixed micelles form as well as antiseizure activity was also reduced.

The perspectives of further investigations of antiseizure effectiveness of nanoparticles should include models of chronic epilepsy, namely pentylenetetrazol (PTZ)-induced kindling, which resembles main features of clinical forms of epilepsy [3].

Hence, **aim** of the investigation was confined to the investigation of the effects of silver nanoparticles upon seizures, induced in PTZ-kindled rats.

Material and Methods of Investigations

Experiments were performed on male Wistar rats (180–270 g). They were kept under standard laboratory conditions, i. e. constant temperature of 23°C, 60% relative humidity, 12-h dark/light cycles, standard diet and tap water was present *ad libitum*. Procedures involving animals and their care were conducted according to Odessa National Medical University ethical committee guidelines that comply with international laws and policies [European Community Council Directive 86/609, OJ L 358, I, December 12, 1987; National Institute of Health Guide for Care and Use of Laboratory Animals, US National Research Council, 1996].

Registration electrodes were implanted under Nembutal anesthesia ("Ceva", France, 40 mg/kg, i. p.): two in frontal and two in occipital regions in both hemispheres (coordinates: AP=1,2; L=3,0; H=1,0 and AP=7,8; L=3,0; H=1,0 correspondently) and into ventral hippocampus (AP=-4,3; L= 4,5; H= 8,0) [6]. Indifferent electrode was placed in nasal bones. Electrodes were fixed to the skull with dental cement. Starting one week after surgery, the rats were handled daily and adapted to the experimental setup. Monopolar EEG registration was started on 7th–14th day from the moment of operation on "DX-5000" computer electroencephalograph (Charkov, Ukraine).

Kindling was induced in rats using a subthreshold dosage of PTZ (30.0 mg/kg, i. p.) ("Sigma Aldrich", USA) starting on the 10th–14th day following the surgery. The total of 21 injections with the epileptogen was carried out. Those animals, which demonstrated generalized clonic-tonic seizures as a response to each of the last three times of PTZ administration, were used for further observation. Testing of behavioral reactions was conducted at 9:00 a. m.–12:00 p. m., 24 hours and three weeks after the last kindling administration of PTZ — early and postponed period correspondently.

Hence, the following groups of rats were formed:

Control group — animals with PTZ-induced kindling treated i. p. with 0.9% saline solution (10 rats);

Second control group — kindled with PTZ and treated with 2.0% colloid solution of ionized argentum (30 rats);

Third group — kindled with PTZ and treated with silver nanoparticles (30 nm) which have been got via citrate method [1, 2] (10 rats).

Thus, the synthesis of silver nanoparticles was performed under the next parameters:

— equimolar concentrations of $AgNO_3$ and $Na_3C_6H_5O_7$: 5x10 — 4 M;

— the ratio of concentrations of $AgNO_3$ to $Na_3C_6H_5O_7$ was 1:4;

— temperature of synthesis was — 100°C;

— period of synthesis was 60 min.

The verification of the size of nanoparticles was performed using optic methods (laser correlation spectroscopy, optical absorbance) and revealed the average value of 30 nm.

ED_{50}, of PTZ which were able to induce clonic seizurtes in 50% of experimental animals have been determined during early and postponed periods of kindling; and the same indices under conditions of nanoparticles administration were verified as well.

Values were compared using one-way analysis of variance followed by a *post hoc t*-test. Values are presented as mean ± standard error of the mean, with findings of $P<0.05$ considered significant.

Results and Discussion

The determination of the dosage of PTZ, which effectively induced seizures in 50% of kindled rats (ED_{50}) was performed in kindled animals in 24 h from the moment of last epileptogen administration. It was established that after PTZ injection in a dosage of 20.0 mg/kg clonic seizures were registered in 3 out of 12 rats, and in two rats the generalized seizure fits have been precipitated later on. The increasing of the PTZ dosage up to 25.0 mg/kg was followed by clonic seizures development in 8 out of 10 kindled rats, and in 5 out of them generalized seizure fits were registered. Hence, ED_{50} of PTZ was 22.0 mg/kg.

Injection of PTZ in the dosage of 15.0 mg/kg, i. p. in postponed period of kindling (three weeks from the moment of last PTZ administration in a dosage of 30.0 mg/kg, i. p.) was followed by clonic seizures development in half of experimental animals (4 rats). It should be stressed that in 3 out of 4 those rats the generalized seizure fits have been precipitated. Higher dosage of PTZ (25.0 mg/kg, i. p.) induced seizure reactions in more than 90% of kindled animals, and prevalent number of them (8 out of 10) demonstrated generalized clonic-tonic fits. Hence, ED_{50} of PTZ in postponed period of kindling was 16.0 mg/kg. This value was reduced by 27.2% when compared with such one, which was observed at the beginning of kindling modeling ($P<0.05$).

ED_{50} of PTZ which induced clonic seizures in 50% animals at the early stage of kindling, which was recalculated after administration of ionized and nanoparticle forms of Ag were less when compared with such one determined in the control group by 13.5% ($P<0.05$) and 26.0% ($P<0.05$) correspondently (Fig. 1). In the postponed period of kindling ED_{50} of PTZ which induced clonic seizures in 50% animals at the early stage of kindling, which was recalculated after administration of ionized and nanoparticle forms of Ag were less when compared with control by 20.0% ($P<0.05$) and by 42.0% ($P<0.05$) (Fig. 1). There was significant reduction of investigated index in comparison with ionized Ag treated rats ($P<0.05$).

EEG investigations revealed the appearance of spike-wave complexes with amplitude of 150–250 mcV and frequency of generation 7 — 11/s in 5.0–7.5 min from the moment of PTZ administration in a dosage of ED_{50} (22,0 mg/kg, i. p., 24 h from the last kindled injection of epileptogen). During next 5.0–10.0 min spike epileptic discharges were registered, which had an amplitude from 0.2 up to 0.7 mV and frequency of generation from 15 to 30 per min (Fig. 2, *a*). Those discharges correlated with clonic seizures.

The analogous administration of PTZ (22.0 mg/kg, i. p.), but after administration of nanoparticles of Ag, was followed in 2.5–5.0 min by appearance of spike potentials with amplitude of 200–500 mcV and frequency

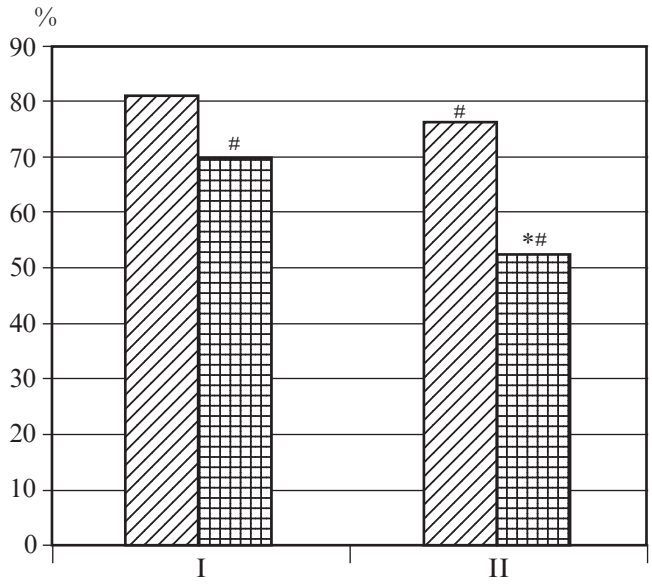

Fig. 1. ED_{50} of PTZ which is able to induce clonic seizures in 50% of experimental animals in kindled animals treated with different forms of Ag. Abscissa: I — ED_{50} determined in 24 h from the last kindled seizure; II — ED_{50} in three weeks from the last kindled seizure. Ordinate: ED_{50} in % pertained to it's value in control group (kindled rats treated with saline solution — 100%). # — P<0,05 when compared with the control group; * — P<0,05 when compared with the group of rats treated with ionized Ag

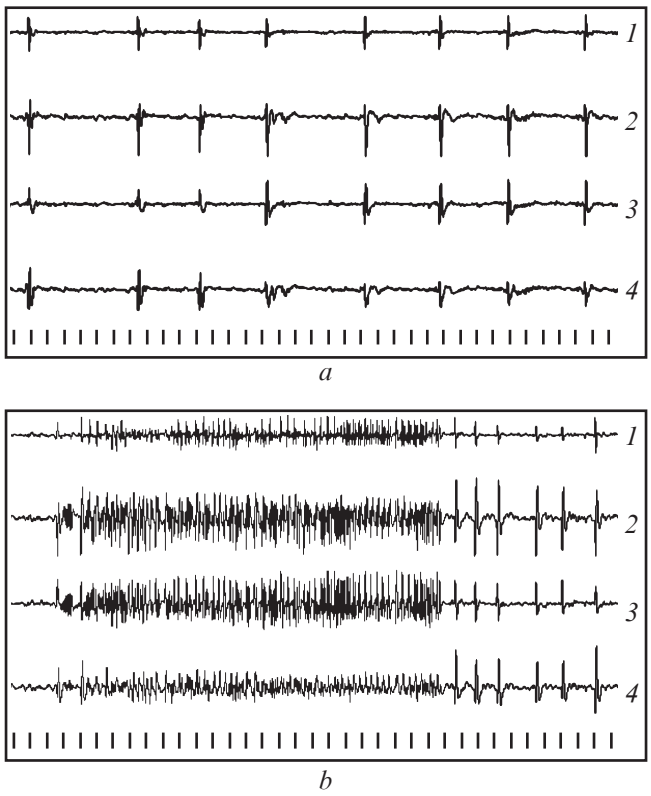

Fig. 2. Eletcrographic manisfestations in rats treated with silver nanoparticles

of generation 15–25 per min. Their amplitude and frequency raised during next 10 min — up to 1.0–1.2 mV and 3/s correspondently (Fig. 2, *b*). Such discharges were registered in all investigated brain structures during next 20.0–40.0 min of observation. The generalized clonic-tonic seizure fits were registered during such synchronized EEG activity with postseizure depression. 3 out of 9 rats died as a result of repeated seizure fits.

Hence, gained data revealed that under conditions of PTZ-induced kindling the net increasing of epileptic activity is observed after administration of silver nanoparticles. Intensification of seizures is registered in the form of decreasing of ED_{50} dosage of PTZ, which caused the clonic seizures in 50% of kindled animals. The effect of facilitation of seizures was more pronounced in postponed kindled state, which is known as resistant and more severe form of chronic brain epileptisation [3]. Administration of silver nanoparticles reduced ED_{50} of PTZ by 42.0%, and this reduction significantly (by 22,0%) exceeded the reduction which was observed in rats treated with ionized form of Ag.

It is also of interest to note that ionized Ag was also able to cause subtle proepileptogenic effects in postponed kindled period, which might be explained by the presence of some amount of nanoparticle form of Ag in colloid Ag solution [1].

It should be stressed, that possible increasing of penetration of blood-brain barrier, as a result of silver nanoparticle effects which underlay facilitative action on PTZ seizures, might be used for better delivering antiepileptic drugs to brain tissue.

Conclusions

Silver nanoparticles are able to cause facilitation of seizures in PTZ-kindled rats, and this effect is more pronounced during postponed stage of kindling development.

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Submitted 14.04.2017
Reviewer R. S. Vastyanov, MD, PhD, prof.