The purpose of the work is to study the chronic convulsing activity intensity in conditions of picrotoxin-induced kindling and due to recombinant antagonist of interleukin-1 receptors introduction as well as registration of electrographic activity of brain structures and investigation of electrical potentials generated by subcortical structures in conditions of recombinant antagonist of interleukin-1 receptors administration. The experimental data are given showing the anticonvulsive action after recombinant antagonist of interleukin-1 receptors intraportaline, intracerebral administrations. Recombinant antagonist of interleukin-1 receptors intrahippocampal, intranigral and intracerebroventricular microinjections resulted in anticonvulsive efficacy expressed mainly by decrease of latency time and number of seizures. The most pronounced anticonvulsive effects were obtained after recombinant antagonist of interleukin-1 receptors intracerebroventricular microinjection which additionally characterized by the first seizure reactions latency increase and the number of rats with tonic-clonic seizures decrease. Less expressed anticonvulsive efficacy was registered in case of recombinant antagonist of interleukin-1 receptors intraperitoneal, intracerebral administrations. Recombinant antagonist of interleukin-1 receptors intrahippocampal, intranigral and intracerebroventricular microinjections resulted in anticonvulsive efficacy expressed mainly by seizure intensity decreasing. The most pronounced anticonvulsive effects were obtained after recombinant antagonist of interleukin-1 receptors intracerebroventricular microinjection which additionally characterized by the first seizure reactions latency increase and the number of rats with tonic-clonic seizures decrease. Less expressed anticonvulsive efficacy was registered in case of recombinant antagonist of interleukin-1 receptors intraperitoneal and intracerebral administrations in conditions of experimental chronic epileptogenesis.

Key words: kindling, ventral hippocampus, black substance, cerebral ventricles, recombinant interleukin-1 receptors antagonist, anticonvulsive effect.

The work is a fragments of the research project “Development of new therapeutic and prophylactic methods and pathogenetic background of their use in inflammatory periodontal diseases together with metabolic syndrome”, state registration No. 0120U0021970.

Due to the circulation in the body of members of the family of pro- and anti-inflammatory cytokines, the latter are considered modulators of CNS activity. It is known that during the first minutes after the initiation of convulsive activity, proinflammatory cytokines and growth factors are released from the brain tissue, one of the representatives of which is interleukin-1-beta (IL-1β) [10, 14]. IL-1β has been shown to alter brain reactivity to convulsive effects, modulate the severity of convulsive reactions, and induce proconvulsive effects [11, 13, 15]. Blockade of the activity of receptors to which IL-1β binds causes anti-inflammatory [7], neuroprotective [6] and anticonvulsant effects in acute and chronic forms of convulsive activity [12].

Inhibition of acute generalized and chronic convulsive activity in the blockade of interleukin-1 (IL 1) receptors due to the use of recombinant antagonist interleukin-1 receptors (RAIL) has been shown [1, 2]. There is also a delay in the formation of picrotoxin (PCT)-induced kindling [4]. Of interest is the study of the effect of blockade of IL-1 receptors under the conditions of a kindling-induced model of epileptogenesis.
The purpose of the work was to study the chronic convulsive activity intensity in conditions of picrotoxin-induced kindling and due to recombinant antagonist of interleukin-1 receptors introduction as well as registration of electrographic activity of brain structures and investigation of electrical potentials generated by subcortical structures in conditions of recombinant antagonist of interleukin-1 receptors administration.

Materials and methods. The experiments were performed under the conditions of a chronic experiment on male Wistar rats weighing 180-250 g. Work with experimental animals was carried out in accordance with domestic and international guidelines for the use of laboratory animals in experimental studies (Council of Europe Convention, 1986; Law of Ukraine “On Animal Protection from ill treatment” dated 21.02.2006, No. 3447-IV), as well as the commission on bioethics of ONMedU.

To reproduce the chronic convulsive syndrome, a model of chemical kindling was used, which was reproduced by 24-day administration of picrotoxin (PCT; “Sigma-Aldrich”, Germany) at a subthreshold dose in the range from 0.9 to 1.1 mg/kg [2].

Kindling rats (a day after the last, 24th PCT injection) were injected through pre-implanted cannulas in the coordinates of stereotactic atlas [9] with a recombinant antagonist of interleukin-1 (RAIL; Scientific-Research Institute of especially pure drugs, St. Petersburg, Russia) in the brain (anterior-posterior [AP]= -0.8; lateral [L]= 1.5; height [H]= 3.5), ventral hippocampus (AP = -4.8; L = 4.5; H = 7.0) and the reticular part of the substantia nigra = -4.8; L = 2.5; H = 8.0) with a volume of 2.0 µl at a dose of 10 and 20 µg.

In separate series of the experiment RAIL-1 was administered intraperitoneally to rats at doses of 2.5, 5.0, 7.5 and 10.0 mg/kg 30 min before the PCT administration.

After 30 min, the rats were placed in individual transparent plastic chambers (10 cm per 25 cm per 30 cm), injected with PCT at a dose of 2.0 mg/kg and recorded the severity of convulsive reactions on a 6-point scale [5]. The latent period of the first convulsive reactions was also estimated. The number of rats with generalized clonic-tonic seizures was also counted. In each experimental group there were 6 rats, in the control (intracerebral injections of 0.9% saline to kindling animals) – 18 rats.

To record the electroencephalographic activity of the brain under ketamine anesthesia in the coordinates of stereotactic atlas [10] rats in the frontal cortex and hippocampus were bilaterally implanted with constantan electrodes in lacquer isolation with a tip diameter of 0.10-0.15 mm, which were fixed to the bones.

EEG in rats began to be recorded on average 6-8 min after PCT. To evaluate the EEG, we used a polling frequency of 256 pulses/s using an ADC (“National Instruments”, USA) – the data were visualized on the screen and recorded on the hard disk for further off-line processing. The frequency range of the signals was 0.5-40 Hz. The frequency ranges were classified as follows: 0.5-4, 4-8, 8-12, 12-25, 25-40 Hz. 16-second EEG recording epochs were subjected to Fourier analysis (Labview-5.0, USA), excluding EEG areas containing artifacts based on visual analysis. recording channels, which we used to on-line assess the time of onset, peak and remission of spontaneous seizures.

The obtained results were calculated statistically using the parametric ANOVA criterion, which was accompanied as a correspondence by the Newman-Kulls criterion, and the nonparametric Kruskal-Wallis test. The minimum statistical probability was determined at p<0.05.

Results of the study and their discussion. Intraperitoneal RAIL administration to kindling rats at a dose of 2.5 mg/kg caused the formation of acute generalized seizures in 6 rats out of 6 in the group, while in 2 animals these attacks were recurrent. The intensity of convulsive reactions and the average interval of the latency period did not differ from similar indicators in rats of the control group (p>0.05, Table 1). A similar situation was observed in the study of PCT-induced convulsive activity under conditions of intravenous administration of RAIL at a dose of 5.0 mg/kg (Table 1). After administration of RAIL-1 at a dose of 7.5 mg/kg, the severity of PCT-induced seizures in kindling rats was comparable to those in the control group (p>0.05). The latency period of these seizures by 32.7% exceeded the corresponding figure in the control observations (p<0.05, table 1).

The intensity of seizures in kindling rats after administration of the maximum (10.0 mg/kg) dose of RAIL was less than in the control (p<0.05, table 1). The latent period of the first convulsive manifestations under such conditions was equal to 24.0±2.3 min, which was 56.9% higher than the corresponding control indicator (p<0.01).

The amplitude-frequency analysis of EEG waves in this series of experiments revealed the predominance of the activity of delta waves, which at the introduction of RAIL minimum dose were most pronounced in the hippocampus (fig. 1, A). However, the power of all frequency rhythms was significantly less than under background registration (p<0.05).

The activity of electrical activity of the τ-range in the studied brain structures averaged 18.1±2.4%, α-range – 9.8±1.0%, β-range – 12.1±1.7% and γ-range – 6.3 ±0.8% compared with the ascending background, which had a discrepancy in the statistical calculation (p<0.05).
### Table 1

<table>
<thead>
<tr>
<th>Rats, RAIL doses, number of rats</th>
<th>Number of rats with seizures intensity</th>
<th>P, compared to control</th>
<th>Latency of the first seizures, M±m, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n=18</td>
<td>0</td>
<td>1 2 3 4 5</td>
<td>-</td>
</tr>
<tr>
<td>Intrapерitoneal administrations:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAIL (2.5 mg/kg), n=6</td>
<td>0</td>
<td>0 0 0 4 2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RAIL (5.0 mg/kg), n=6</td>
<td>0</td>
<td>0 0 0 5 1</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>RAIL (7.5 mg/kg), n=6</td>
<td>0</td>
<td>0 0 1 5 0</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>RAIL (10.0 mg/kg), n=6</td>
<td>0</td>
<td>0 1 1 4 0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intracerebroventricular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>administrations:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAIL (10 μg), n=6</td>
<td>0</td>
<td>0 2 2 0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RAIL (20 μg), n=6</td>
<td>0</td>
<td>0 4 1 1 0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intraperitoneal administrations:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAIL (10 μg), n=6</td>
<td>0</td>
<td>0 0 1 5 0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RAIL (20 μg), n=6</td>
<td>0</td>
<td>0 2 2 2 0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intrahippocampal administrations:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RAIL (10 μg), n=6</td>
<td>0</td>
<td>0 0 0 4 2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RAIL (20 μg), n=6</td>
<td>0</td>
<td>0 1 2 3 0</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Notes: seizure intensity significant dereferences were calculated using Kruskall-Walls criteria; * - p<0.05, ** - p<0.01, *** - p<0.001 - significant dereferences of the investigated parameter compared with the same in kindling rats (ANOVA criteria).

When RAIL was administered at a dose of 5.0 mg/kg under conditions of kindling seizures in all studied brain formations on the EEG of this rat mainly synchronized high-amplitude activity with a frequency of potentials up to 7-9 per min was observed (fig. 1, B). Amplitude-frequency analysis of EEG activity at this time revealed a significant increase in wave power in rats, mainly in the α-, τ- and δ-ranges (fig. 1, B). In all cases, the power generated by the left and right hippocampus was significantly higher compared to those in the right and left frontal cortex. To the greatest extent – by 69% and 59% – increased the power of α- and τ-waves generated by the left hippocampus, which exceeded the corresponding ascending values (p<0.05). In the cerebral cortex, the power of τ- and α-band waves also increased significantly when compared with their ascending values (p<0.05; fig. 1, B).

![Fig. 1. EEG waves magnitude in rats brain in conditions of picrotoxin-induced kindling after RAIL intraperitoneal administration.](image)

Notes: A-D corresponds to RAIL different doses; * - p<0.05 – significant differences pertaining the initial data.

Convulsive manifestations and their EEG-correlates in the case of introduction of RAIL to kindling rats at a dose of 7.5 mg/kg were highlighted in such a way that the EEG significantly increased the amplitude of adhesion potentials generated with a frequency of 11-13 per min in both hippocampi (fig. 1, B). In the neocortex, the potential frequency was 8-9 per minute. The amplitude of the potentials in the frontal cortex
and hippocampus was 1.3-1.7 mV. The duration of convulsive activity averaged 50-70 s and was characterized by the appearance of high-frequency adhesion activity in the EEG, which could be observed independently in only one hippocampus or both simultaneously with the discharges in the neocortex. This was confirmed by a significant increase in the power of the $\delta$- and $\alpha$-ranges, which in this case were also maximum in the left hippocampus (92% and 95% more than at the beginning of registration; $p<0.05$). The total power data of cortical neurons on the left and right sides are quite high – on average 162.3±17.4% and 157.5±16.4% ($p<0.05$). In this time interval, the manifestation of pilocarpine-induced seizures is accompanied by inhibition of the activity of the $\tau$-, $\beta$-, and $\gamma$-ranges ($p<0.05$; fig. 1, B).

EEG manifestations after RAIL administration at the maximum dose were marked by EEG transformation: this variant of the electrical activity of the brain was dominant in 2 rats out of 6 (33.3%). In some rats, high-amplitude EEG activity associated with the development of convulsive activity, within 1 min was transformed into acute adhesion potentials with a frequency of 11-13 per min and an amplitude of 1.6-1.7 mV (fig. 1, D), which behaviorally corresponded to small-amplitude contractions muscles of the muzzle, head and forelimbs (2 points on the accepted scale of seizure severity).

Frequency-amplitude analysis of the EEG in these experimental conditions revealed a predominance of $\delta$- and $\alpha$-activity in the hippocampus and cerebral cortex ($p<0.05$; fig. 1, D). The wavelengths of the $\tau$-, $\beta$-, and $\gamma$-bands were minimal, which was less than before the PCT introduction ($p<0.05$).

The first convulsive PCT-induced reactions in rats 24 h after the last convulsant injection developed after 15.3±1.4 min and were predominant generalized clonic-tonic seizures (11 rats out of 18) including recurrent ones (5 rats from 18), with the development of post-attack depression and autonomic disorders (table 1). Intraventricular administration of RAIL (10 µg) was characterized by the development of generalized seizures in only 2 rats out of 6 ($p<0.05$). The latency of the first seizures under such conditions increased by 74.5%, which significantly exceeded the corresponding figure in the control group ($p<0.001$, table 1). PCT-induced seizures in kindling rats after intracerebral administration of RAIL at twice the dose were similar.

Intrahippocampal RAIL (10 µg) administration did not change the severity of PCT-induced convulsive reactions compared with the corresponding initial values ($p>0.05$). Picrotoxin-induced seizures in kindling rats after intrahippocampal administration of RAIL at a dose of 20 µg were characterized by the development of generalized seizures in 2 animals out of 6, which in the number of animals with generalized seizures and the intensity of seizures was less than in control (in both cases $p<0.01$). The latency of the first seizures was half that of kindling rats with intrahippocampal injection of 0.9% saline.
The severity of PCT-induced seizures in kindling rats after RAIL intranigral administration at a dose of 10 µg was comparable with the following indicators in the control group (p>0.05). The intensity of seizures in kindling rats in the case of intranigral administration of RAIL at a dose of 20 µg was less than in the control (p<0.05, table 1) and less than in kindling rats that got injected with RAIL (p<0.01). Generalized seizures under these conditions were registered in 3 rats from 6 corresponding indicators in the control group (p<0.05). The latency of seizures did not change significantly (p>0.05) compared with kindling rats which were i.p. injected with saline.

Analysis of the amplitude-frequency characteristics of EEG recording under conditions of intranigral administration of RAIL revealed that under conditions of activity in the brain of kindling rats (without RAIL) revealed a predominance of α-activity power (on average 27-45% more than the ascending data), which was generated by cortical neurons (p<0.05; fig. 3, A). The power of δ- and τ-range waves did not differ significantly from the corresponding indicators of hippocampal neurons (p>0.05).

The wavelength of the α-band of cortical neurons was 29% higher than the average power of the α-band of hippocampal neurons (p<0.05). The wavelength of the τ-band waves of cortical neurons was 37% higher than the average wave power of the δ-band neurons of the hippocampus (p<0.05). When comparing the power generation of τ-band waves by neurons of the cerebral cortex and hippocampus, the differences were insignificant (p>0.05; fig. 3, A).

In the case of RAIL intranigral administration to kindling rats with a minimum dose the amplitude-frequency analysis revealed an even greater predominance of the activity of the α- (on average 74-88% more) and δ- (on average 67-78% more than the ascending data) ranges, which was generated by cortical neurons in case of PCT-induced seizures (p<0.05; fig. 3, B).

The power predominance of α- and δ-rhythms of cortical neurons in comparison with such indicators in hippocampal neurons was established by frequency-amplitude analysis of EEG during its registration after intranigral administration of RAIL to kindling rats at a dose of 20 µg (fig. 3, B).

The data obtained cover a wide range of development of anticonvulsant action under conditions of blocking the activity of IL-1 receptors. The possibility of suppressing the severity of convulsive activity in the case of the blockade of cytokine receptors under conditions of intracerebral administration of RAIL in addition to the shown effect of inhibition of acute generalized, kindling-induced convulsive activity and that of post-kindling in the case of systemic administration of RAIL.

Our results indicate the maximum severity of the anticonvulsant effect of blocking IL-1 receptors in kindling rats after intravenous administration of the test compound, the rapid development of which can be explained by the direct admission of RAIL and rapid blocking of the activity of relevant cytokine receptors. The relatively lower efficiency of intrahippocampal and intranigral administration of RAIL is explained by the need of the compound (after its introduction into the corresponding brain formation) to bind to the appropriate types of cytokine receptors, which are few or absent [8], or the need to spread it to other brain formations,
which requires additional time, the presence of transport processes, etc., which ultimately significantly limits the prospect of anticonvulsant effect in the case of blocking the activity of cytokine receptors.

The registered anticonvulsant effect in the case of blocking the functional activity of IL-1 receptors is manifested by a decrease in the intensity of pharmacocorrection-resistant kindling seizures, a decrease in the number of animals with generalized clonic-tonic seizures and an increase in the latent period of the first convulsive reactions. Importantly, the protective effect developed after intrahippocampal administration of RAIL and was minimally expressed after intranigral administration of the compound. This demonstrates the possibility of direct blockade of IL-1 receptors in the ventral hippocampus and perihippocampal region, which is fundamental, given the determinant role of the hippocampus in the pathogenesis of chronic convulsive activity [3].

The electrographic activity of brain formations under conditions of kindling seizures in the case of different routes of RAIL-1 suggests that one of the main criteria that explain the anticonvulsant effects of RAIL-1 in these conditions of chronic EpA is the inhibition of electrical activity in the hippocampus and frontal cortex with some advance in the hippocampus.

Thus, the development of anticonvulsant action after intracerebral – mainly intracerebroventricular and intrahippocampal – RAIL injections in rats with PCT-induced kindling adds a complete picture of the effects of blockade of IL-1 receptor activity. Under these conditions, anticonvulsant action is realized in acute generalized seizures with different mechanisms of initiation of convulsive effects [1]. Systemic administration of RAIL delays the development of chronic convulsive activity in picrotoxin kindling, inhibits convulsive activity under conditions of kindling and postkindling [4]. All the above effects of RAIL are an experimental justification for testing the clinical efficacy of IL-1 receptor blockade in patients with resistant forms of epilepsy. Prospects for further development, in our opinion, are to identify possible mechanisms for the implementation of anticonvulsant activity of RAIL or to elucidate certain brain formations, the modulation of the functional activity of which affects the antiepileptic activity of the test compound.

Conclusions

1. Intracerebral injections of RAIL cause the development of anticonvulsant effect in terms of kindling-induced chronic convulsive activity.

2. The most pronounced anticonvulsant effect was achieved by intraventricular administration of RAIL. A relatively less pronounced anticonvulsant effect was recorded with intrahippocampal administration of the compound. The lowest efficiency was achieved with the introduction of RAIL in the reticular part of the black substance.

3. The noted anticonvulsant effect after intracerebral administration of RAIL was characterized by a decrease in the intensity of kindling seizures, a decrease in the number of animals with generalized clonic-tonic seizures and an increase in the latency of the first convulsive reactions.

4. One of the mechanisms of realization of anticonvulsant efficiency of RAIL-1 in the conditions of kindling-induced chronic convulsive activity is the suppression of initiation and distribution of electrical activity in the hippocampus and frontal cortex – brain formations, which are considered determinants of the epileptic syndrome.

References


The purpose of the investigation was to study the effect of Plasmogel from platelet autoplasm on the regeneration of collagen fibers of the periodontal ligament in an experimental study on rats with a ligature model of periodontitis. The animals were divided into 2 groups: the first – control of the healing of the pathology without treatment; the second – experimental. For morphological examination, tissue biopsies were taken. Mallory staining was used to determine the fibrous structures of connective tissue. On visual inspection, healing occurred in both groups. However, it didn't go the same way. In Group I, visual recession was noted. In Group II, the largest area occupied by collagen fibers in the periodontal ligament was recorded in Group II of animals, which is 61.93%. In Group I animals, indicator of the area of collagen fibers significantly lower – 35.44%. Plasmogel is used to create a framework and microenvironment for the growth of collagen fibers of the periodontal ligament, which increases the regenerative capabilities.

**Key words:** biomaterials, periodontitis, ligature model, plasma therapy, collagen fibers.

The work is a fragment of the research project: “Correction of pathogenetic mechanisms of metabolic disorders in the oral cavity tissues in patients depending on environmental and alimentary factors affecting carbohydrate and lipid metabolism”, state registration No. 0118U006966.

In recent decades, generalized periodontitis occupies a leading position in the structure of dental morbidity. Periodontitis, one of the diseases of the oral cavity that has a high prevalence among all segments of the population. Severe periodontitis destroys periodontal tissues and leads to teeth loss [10], a sharp deterioration in the quality of life and disorders of general health. Patients with a history of periodontitis have a high risk of developing various systemic diseases, such as diseases of the cardiovascular system, cancer, and metabolic disorders [6, 9, 11]. Proceeding from the above, the search for effective and safe methods of periodontitis treatment is one of the urgent tasks of modern dentistry.

The development of therapy methods for the regeneration of periodontal tissues, including the alveolar bone, root cementum and the periodontal ligament continues [5]. At the moment, the “gold” standard of treatment is a multidisciplinary integrated approach. The main part of non-surgical treatment procedures is aimed at eliminating the etiological microbial factor (professional oral hygiene, smoothing the root surface, teaching individual oral hygiene, etc.). However, with the removal of periodontal pathogens and pathological tissues, only a small amount of periodontal tissues can be restored [10]. Using more complex surgical methods...