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## LOW-DOSE DIGOXIN ENHANCES THE ANTICONVULSIVE POTENTIAL OF CARBAMAZEPINE AND LAMOTRIGINE IN CHEMO-INDUCED SEIZURES WITH DIFFERENT NEUROCHEMICAL MECHANISMS

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*“Non-antiepileptic” drugs have a strong potential as adjuvants in multidrug-resistant epilepsy treatment. In previous study the influence of low doses of digoxin, which do not affect the myocardium, on the anticonvulsant potential of classical commonly used anti-epileptic drugs under conditions of seizures, induced by pentylenetetrazole and maximal electroshock, has been investigated.*

**The aim of the study** was to investigate the influence of digoxin at a sub-cardiotonic dose on the anticonvulsant potential of carbamazepine and lamotrigine in experimental seizures with different neurochemical mechanisms.

**Material and methods.** A total of 192 random-bred male albino mice weighting 22–25 g were used. Carbamazepine and lamotrigine were administered intragastrically in conditionally effective ( $ED_{50}$ ) and sub-effective ( $1/2 ED_{50}$ ) doses: carbamazepine at doses of 100 and 50 mg/kg; lamotrigine at doses of 25 and 12.5 mg/kg. Digoxin was administered subcutaneously at a sub-cardiotonic dose of 0.8 mg/kg as an adjuvant to carbamazepine and lamotrigine in  $1/2 ED_{50}$  Picrotoxin (2.5 mg/kg subcutaneously); thiosemicarbazide (25 mg/kg intraperitoneally); strychnine (1.2 mg/kg subcutaneously); camphor (1000 mg/kg intraperitoneally) were used as convulsant agents.

**Results.** It was found that digoxin not only has its own permanent anticonvulsant effect on different models of paroxysms with different neurochemical mechanisms of development, but also significantly enhances the anticonvulsant potential of carbamazepine (to a lesser extent – lamotrigine) regardless of the pathogenesis of experimental paroxysms.

**Conclusion.** Based on the results, it can be concluded that digoxin has a high potential as an adjuvant medicine in complex epilepsy treatment because it enhances the efficiency of low-dose traditional anticonvulsants carbamazepine and lamotrigine

**Keywords:** anti-epileptic drugs, digoxin, adjuvant, chemo-induced seizures, mice

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### 1. Introduction

Despite a wide range of modern antiepileptic drugs (AEDs), most of which also have multiple mechanisms of influence on the development of seizures [1, 2], every fourth patient with epilepsy is multidrug-resistant [3, 4]. In parallel with the development of the latest AEDs, the search for promising correctors of refractory epilepsy among drugs of different pharmacological groups – the so-called “non-antiepileptic” drugs (non-AEDs) [5]. Among such drugs, antiarrhythmic drugs deserve the highest attention – sodium, potassium and calcium channel blockers, as well as cyclooxygenase inhibitors, interleukin antagonists, angiotensin II receptor blockers, etc. [5–8]. The effect of such drugs on certain links in the epilepsy pathogenesis is exactly unknown. The authors suggest that their anticonvulsant effect may be associated with blockade of transmembrane currents of sodium, potassium and calcium (particularly for antiarrhythmic drugs of I-IV classes according to the Vaughan-Williams classification), or reduction of neuroinflammation (for cyclooxygenase inhibitors, interleukin antagonists), etc. It has been found, however, that

the anticonvulsant activity of non-AEDs *per se* is moderate, but the addition of such drugs to traditional – classical – AEDs in various regimens for refractory epilepsy allows to achieve much more stable disease control [9]. In addition, antiepileptic drugs have their own side effects (including AV blockade or sinus blockade for antiarrhythmic drugs, ulcerogenic effect for cyclooxygenase inhibitors, hypotension for angiotensin II receptor blockers), which justifies the advisability of further studies of their use in adjuvant therapy of epilepsy.

One such non-AEDs with previously experimentally and clinically proven anticonvulsant activity is the known cardiac glycoside digoxin at a sub-cardiotonic doses that do not cause side effects [10, 11]. We have previously investigated the effect of low doses of digoxin, which do not affect the myocardium, on the anticonvulsant potential of seven classical commonly used AEDs with different mechanisms of influence on the molecular mechanisms of epileptogenesis [12, 13]. It was found that in the basic models of primary generalized seizures – paroxysms, induced by pentylenetetrazole and maximal electroshock – digoxin in a sub-cardiotonic dose signifi-

cantly potentiates the anticonvulsant action of valproate, carbamazepine, levetiracetam, lamotrigine, topiramate, clonazepam and phenobarbital [12, 13]. There is, however, the question of an in-depth study of the effect of digoxin on various neurochemical links in the development of seizures, as well as the possible modulation of molecular receptor mechanisms and, as a consequence, the anticonvulsant action of classical AEDs.

Therefore, the aim of this work was to investigate the influence of digoxin at a sub-cardiotonic dose on the anticonvulsant potential of carbamazepine and lamotrigine in experimental seizures with different pathogenesis.

## 2. Planning (methodology) of the research

The methodology was designed as following Fig. 1 according to the aim of the research.

The research was performed using mice as a convenient universal test system in the study of anticonvulsant effect of drugs [14].

The models of chemo-induced seizures were chosen taking into account different molecular mechanisms of convulsions development [15] and, as a consequence, the ability to establish the neurochemical links of the anticonvulsant action realization for digoxin and its combinations with carbamazepine and lamotrigine. Unlike the basic models of pentylenetetrazole-induced seizures and maximal electroshock, which are used for the initial screening of compounds, these models are used by the court for in-depth study of the anticonvulsant action of promising anticonvulsant drugs [15, 16]. Carbamazepine and lamotrigine have been selected as widely used drugs for first-line treatment of epilepsy [1, 2, 5].

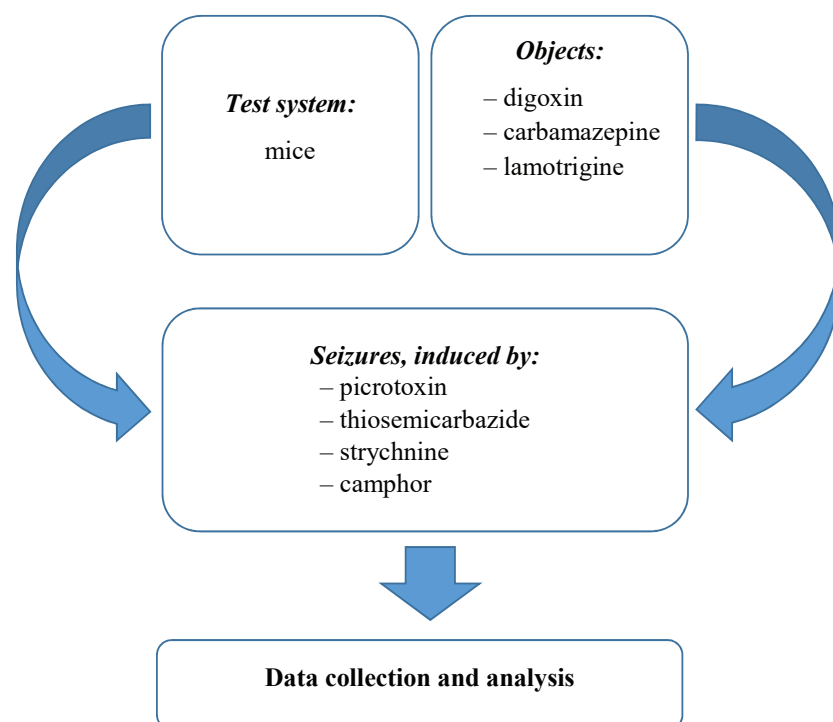


Fig. 1. Algorithm of the research

## 3. Material and methods

Experiments were conducted from February to March 2021.

192 random-bred male albino mice weighting 22–25 g were used in the experiments. Animals were kept in standard laboratory conditions at constant humidity and temperature +18–20 °C, food and water ad libitum, in the Central Research Laboratory of Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy (Kharkiv, Ukraine). The experiments were performed in accordance with bioethical principles and standards in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and Council of Europe Directive 2010/63/EU (2010). All experiments have been approved by the Bioethics Commission of the National University of Pharmacy (protocol No. 3 from 10 September 2020).

The anticonvulsant effect of digoxin and its combinations with AEDs was studied under conditions of picrotoxin-, thiosemicarbazide-, strychnine- and camphor-induced seizures [15, 16]. Animals were randomly divided into groups of 6 mice each: 1 group – control (untreated seizures), the remaining groups – animals with model seizures, which were administered carbamazepine, lamotrigine, as well as their combinations with digoxin.

Classical AEDs were administered once intragastrically (i.g.) as an aqueous suspension stabilized with tween-80, in conditionally effective ( $ED_{50}$ ) and sub-effective ( $1/2 ED_{50}$ ) doses 30 min before the administration of a convulsive agent: carbamazepine (Finlepsin, Teva Operations Poland) – at doses of 100 and 50 mg/kg; lamotrigine (Lamictal, GlaxoSmithKline Pharmaceuticals, Poland) – at doses of 25 and 12.5 mg/kg [17]. This choice of doses is justified by the necessity to determine the possible potential effects of digoxin on the anticonvulsant action of drugs. Digoxin (Zdorovye, Ukraine) was administered once subcutaneously (s.c.) at a previously studied effective anticonvulsant dose of 0.8 mg/kg (which is equal to  $1/10 LD_{50}$ ) [10] 10–15 minutes before seizure induction. Control animals received i.g. purified water in an appropriate volume (0.1 ml per 10 g of body weight).

According to the model of seizures as convulsants were used: picrotoxin (Sigma, USA) – aqueous solution, 2.5 mg/kg s.c.; thiosemicarbazide (Sigma, USA) – aqueous solution, 25 mg/kg intraperitoneally (i.p.); strychnine (strychnine nitrate, Sigma, USA) – aqueous solution, 1.2 mg/kg s.c.; camphor (Sigma, USA) – oil solution, 1000 mg/kg i.p. [15, 16].

After administration of the convulsive agent, mice were placed in individual transparent plastic cylindrical boxes and the condition of the animals was continuously monitored for 1 hour (in the model of thiosemicarbazide seizures – 4 hours). The severity of anticonvulsant effects of drugs and their combinations was assessed by the following indicators:

latency of the convulsions; the number of clonic-tonic convulsions per one mouse; % of animals with clonic and tonic convulsions; the duration of the convulsive period (from the first to the last convulsion); and the lifetime of the animals before death (in mice with a lethal outcome) were calculated. The severity of seizures was evaluated according to a scale ranging from 1 to 6: 1 – trembling; 2 – circus movement; 3 – clonic seizures; 4 – clonic-tonic seizures with a lateral position; 5 – tonic extension; 6 – tonic extension leading to the death of the animal). If seizures were not observed within 1 h, it was considered that the latency period was 60 min (in the model of thiosemicarbazide seizures – 240 min) [18].

Statistical processing of the obtained results was performed using the software package STATISTICA 12 (StatSoft, USA) with the calculation of the mean value, standard error of mean, the level of statistical significance ( $p$ ). Significance of differences between comparison groups was assessed by parametric Student's  $t$ -test – in cases of normal distribution, non-parametric Mann-Whitney  $U$ -test – in its absence. The results, which were determined in an alternative form (lethality, % of mice with clonic and tonic convulsions) were evaluated using the Fisher's criterion ( $\phi$ ).

#### 4. Results

Polymodal anticonvulsant properties of digoxin *per se* were verified by the pronounced effect of cardiac glycoside on the course of all studied chemo-induced seizures (Tables 1, 2). In the picrotoxin-induced seizure model, digoxin not only significantly reduced lethality – almost 4-fold relative to control ( $p < 0.05$ ), but also statistically significantly increased the latency of the first seizure, reducing the % of animals with clonic and tonic convulsions, severity of paroxysms and duration of seizures. In the thiosemicarbazide-induced seizure model, the anticonvulsant potential of cardiac glycoside was determined both by a statistically significant reduction in animal death (lethality – 67 % vs. 100 % in the control group,  $p < 0.05$ ) and synchronous reduction % of animals with tonic paroxysms and probable prolongation the period of the first seizure. The antagonism of digoxin relative to the convulsive action of strychnine is not only a highly significant ( $p < 0.01$ ) threefold decrease in lethality, but also a statistically significant fourfold increase in the latency time of first seizures ( $p < 0.01$ ), a twofold reduction in the number of convulsion per animal (due to both clonic and tonic component) and the severity of seizures ( $p < 0.05$ ) relative to control. In the camphor-induced seizure model, digoxin completely prevented the death of experimental mice in the group (lethality – 0 %,  $p < 0.01$ ), and causes a probable reduction in the severity of paroxysms relative to similar indicators of the group of animals with untreated seizures.

The results of studying the effect of digoxin on the anticonvulsant effect of carbamazepine are presented in Table 1.

Under paroxysms induced by picrotoxin, the anticonvulsant effect of carbamazepine in both doses is a statistically significant reduction in lethality ( $p < 0.01$ ), as

well as a significant reduction in seizure severity ( $p < 0.05$ ) relative to control. In addition, under the influence of the drug at a  $\frac{1}{2} ED_{50}$  there is a significant decrease in the % of mice with tonic seizures in the group.

The combined use of carbamazepine at a  $\frac{1}{2} ED_{50}$  with digoxin is characterized by complete prevention of animal death (lethality – 0 %, like AED at an  $ED_{50}$ ), statistically significant relative to control ( $p < 0.05$ ) reduce in the severity of seizures and the duration of the convulsive period. The combination of drugs also clearly prolongs the time to the onset of the first seizures – significant not only for control, but also for carbamazepine at both doses ( $p < 0.01$ ).

In the thiosemicarbazide-induced seizure model, carbamazepine has a pronounced dose-dependent effect. Although the reducing effect of AED at both doses on lethality is completely comparable, at  $ED_{50}$  in contrast to a  $\frac{1}{2} ED_{50}$ , carbamazepine more than tripled the period of the first convulsions and halved the % of mice with clonic and tonic seizures ( $p < 0.01$ ), increasing the lifetime of the animals before death ( $p < 0.05$ ).

The addition of digoxin to carbamazepine at a  $\frac{1}{2} ED_{50}$  not only completely prevents thiosemicarbazide-induced animal death (lethality – 0 %,  $p < 0.01$ ), which is statistically significant not only for control, but also for digoxin *per se* and AED in both doses. In addition, the combined use of carbamazepine with digoxin significantly prolongs the latency period of the first convulsions, while reducing the number of animals with clonic and tonic seizures and the severity of paroxysms.

In terms of its effect on the experimental convulsive syndrome caused by strychnine, carbamazepine at a  $\frac{1}{2} ED_{50}$  is slightly inferior to the effects of the drug at a  $\frac{1}{2} ED_{50}$ . Although in both doses AED significantly reduces lethality, prolongs the latent period of the first convulsions, reduce the % of animals with clonic and tonic convulsions and the severity of paroxysms, a more pronounced anticonvulsant effect is still inherent in carbamazepine in a conditionally effective dose. The effect of AED at an  $ED_{50}$  is additionally manifested in a statistically significant reduction in the number of clonic-tonic seizures per one mouse and a highly significant reduction in the duration of the convulsive period ( $p < 0.01$ ).

The efficacy of the combined use of digoxin with carbamazepine at a  $\frac{1}{2} ED_{50}$  in the model of strychnine-induced seizures is comparable to the anticonvulsant effect of classical AED at an  $ED_{50}$ , which is confirmed by the same effect of drugs on all studied indicators of experimental paroxysms.

The anticonvulsant potential of carbamazepine at an  $ED_{50}$  in the camphor-induced seizure model is limited to a statistically significant effect on the number of paroxysms per mouse, the tonic component of seizures, the severity of convulsions, and the duration of the convulsive period. At a  $\frac{1}{2} ED_{50}$  of AED significantly reduces animal lethality (17 vs. 67 % in control,  $p < 0.05$ ), reduces the % of mice with tonic convulsions and the severity of paroxysms.

Table 1  
Anticonvulsant effect of digoxin, carbamazepine and their combination in the primary generalized seizures in mice  
( $n=6$ )

Group of animals	Latency, min	Number of clonic-tonic seizures in 1 mouse	% of mice with convulsions		Severity of seizures, points	Period of seizures, min	Time to death, min	Lethality, %
			clonic	tonic				
<i>Picrotoxin-induced seizures</i>								
Control (untreated seizures)	12.06±0.71	4.83±1.40	100	100	5.33±0.42	29.77±5.93	43.83±9.16	67
Digoxin	41.98±6.48**	1.67±0.61	67*	50**	2.83±0.98*	3.44±1.56**	33.40	17*
CBZ, ED <sub>50</sub>	13.75±0.40 <sup>#</sup>	5.17±1.40	100 <sup>#</sup>	83	3.83±0.17*	17.58±4.55 <sup>#</sup>	–	0**
CBZ, ½ ED <sub>50</sub>	16.41±1.41 <sup>#</sup>	4.33±1.05	100 <sup>#</sup>	67*	3.67±0.21*	17.08±6.43	37.95	17*
CBZ, ½ ED <sub>50</sub> + Digoxin	36.48±4.37**§§°°	4.00±0.89	100 <sup>#</sup>	83	3.83±0.17*	12.95±3.88*	–	0**
<i>Thiosemicarbazide-induced seizures</i>								
Control (untreated seizures)	54.72±2.92	1.83±0.40	100	100	6.00±0.00	7.62±3.88	62.34±4.27	100
Digoxin	75.98±4.75*	1.50±0.34	100	67*	5.00±0.63	5.03±2.95	76.84±3.50	67*
CBZ, ED <sub>50</sub>	172.13±30.76***	2.17±1.28	50***	50**	3.00±1.34	13.14±7.25	130.55±7.09*	50**
CBZ, ½ ED <sub>50</sub>	108.58±28.47	3.00±1.03	83	83	4.33±0.95	35.17±15.42	113.68±28.93	50**
CBZ, ½ ED <sub>50</sub> + Digoxin	161.34±25.20***	1.50±0.62	67 <sup>#</sup>	50**	2.50±0.81**	13.45±7.95	–	0***§§°°
<i>Strychnine-induced seizures</i>								
Control (untreated seizures)	7.73±0.46	1.33±0.21	100	100	6.00±0.00	1.15±0.64	8.88±0.76	100
Digoxin	31.28±9.17**	0.67±0.21*	67*	33**	3.00±1.10*	0.33±0.19	18.55±2.90	33**
CBZ, ED <sub>50</sub>	51.89±8.11**	0.17±0.17**	17***	17**	0.67±0.67**	0.02±0.02**	–	0** <sup>#</sup>
CBZ, ½ ED <sub>50</sub>	33.97±11.73*	0.83±0.40	50**	50**	2.33±1.09**	0.39±0.22	8.71±3.50	33**
CBZ, ½ ED <sub>50</sub> + Digoxin	51.11±8.89*	0.17±0.17**	17***	17**	0.67±0.67**	0.02±0.02**	–	0** <sup>#</sup>
<i>Camphor-induced seizures</i>								
Control (untreated seizures)	4.96±0.75	7.33±1.12	100	100	5.33±0.42	46.26±10.90	40.86±12.62	67
Digoxin	6.22±0.36	5.33±0.42	100	100	4.00±0.00*	44.85±7.42	–	0**
CBZ, ED <sub>50</sub>	16.27±8.83	2.33±0.56 <sup>#</sup>	83	50***	3.00±0.63*	9.09±2.88 <sup>#</sup>	21.32±1.38	33 <sup>#</sup>
CBZ, ½ ED <sub>50</sub>	8.33±1.31	4.67±1.33	100	50***	3.83±0.48*	15.05±4.79 <sup>#</sup>	31.35	17*
CBZ, ½ ED <sub>50</sub> + Digoxin	10,34±1,29 <sup>#</sup>	1,00±0,00***§§°°	100	17***	3.17±0.17***	0.13±0.01***§§°°	10.08	17*

Note:  $n$  – number of animals in each group; CBZ – carbamazepine; \* –  $p < 0.05$  when compared with control; \*\* –  $p < 0.01$  when compared with control; <sup>#</sup> –  $p < 0.05$  when compared with digoxin, <sup>##</sup> –  $p < 0.01$  when compared with digoxin; <sup>§</sup> –  $p < 0.05$  when compared with carbamazepine at an ED<sub>50</sub>; <sup>§§</sup> –  $p < 0.01$  when compared with carbamazepine at an ED<sub>50</sub>; <sup>°</sup> –  $p < 0.05$  when compared with carbamazepine at a ½ ED<sub>50</sub>; <sup>°°</sup> –  $p < 0.01$  when compared with carbamazepine at a ½ ED<sub>50</sub>

The combination of carbamazepine at a ½ ED<sub>50</sub> with digoxin causes a pronounced anticonvulsant effect, which in terms of the effect on individual studied indicators probably exceeds the effect of not only the individual components of the combination, but also AED at an ED<sub>50</sub>. Thus, the combination significantly reduces the lethality of animals in the group ( $p < 0.05$ ), statistically significantly prolongs the time of the first paroxysms, reducing the number of convulsions per one mouse (mainly due to the tonic component), as well as the severity of convulsions and the duration of the convulsive period. Although the effectiveness of the combination of carbamazepine and digoxin under camphor-induced seizures (lethality 17 % or 1/6) seems smaller than against monotherapy of digoxin (lethality 0 % or 0/6), this difference with such a number of animals in the experimental groups has no statistical significance.

The results of the study of the effect of digoxin on the spectrum of anticonvulsant action of lamotrigine are shown in Table 2.

The ambiguity of the effect of lamotrigine on the course of picrotoxin seizures was found, on the one hand, due to the absence of effect of AED at an ED<sub>50</sub>, and on the other – statistically significant prolongation of the latent period of the first convulsion against the background of significant ( $p < 0.05$ ) increase in animal lethality under the action of the drug at an ED<sub>50</sub>.

Combined use of lamotrigine at ½ ED<sub>50</sub> with digoxin, although not affecting the mortality of animals in the experimental group, still causes a probable prolongation of the latent period of the first convulsions and a decrease in % of mice with tonic convulsions, but inferior to the effectiveness of digoxin monotherapy.



Table 2

Anticonvulsant effect of digoxin, lamotrigine and their combination in the primary generalized seizures in mice ( $n=6$ )

Group of animals	Latency, min	Number of clonic-tonic seizures in 1 mouse	% of mice with convulsions		Severity of seizures, points	Period of seizures, min	Time to death, min	Lethality, %
			clonic	tonic				
<i>Picrotoxin-induced seizures</i>								
Control (untreated seizures)	12.06±0.71	4.83±1.40	100	100	5.33±0.42	29.77±5.93	43.83±9.16	67
Digoxin	41.98±6.48**	1.67±0.61	67*	50**	2.83±0.98*	3.44±1.56**	33.40	17*
LTG, ED <sub>50</sub>	15.16±1.89 <sup>#</sup>	6.50±0.56 <sup>##</sup>	100 <sup>#</sup>	100 <sup>##</sup>	5.67±0.33 <sup>#</sup>	21.02±1.71 <sup>##</sup>	34.38±2.22	83 <sup>##</sup>
LTG, ½ ED <sub>50</sub>	16.43±1.58 <sup>##</sup>	4.50±0.56 <sup>#</sup>	100 <sup>#</sup>	100 <sup>##</sup>	6.00±0.00 <sup>##</sup>	21.43±2.73 <sup>##</sup>	37.86±3.60	100 <sup>##</sup>
LTG, ½ ED <sub>50</sub> +Digoxin	23.89±4.70 <sup>**#§</sup>	2.50±0.43 <sup>§§°</sup>	100 <sup>#</sup>	67 <sup>*§°</sup>	5.00±0.63	15.74±4.76 <sup>#</sup>	41.36±3.81	67 <sup>°</sup>
<i>Thiosemicarbazide-induced seizures</i>								
Control (untreated seizures)	54.72±2.92	1.83±0.40	100	100	6.00±0.00	7.62±3.88	62.34±4.27	100
Digoxin	75.98±4.75*	1.50±0.34	100	67*	5.00±0.63	5.03±2.95	76.84±3.50	67*
LTG, ED <sub>50</sub>	107.11±26.98 <sup>**</sup>	5.00±1.26*	83	83	4.50±0.96	63.78±15.45*	144.33±7.97*	50 <sup>**</sup>
LTG, ½ ED <sub>50</sub>	83.57±10.99*	3.33±0.84	100	100 <sup>#</sup>	5.33±0.42	55.27±16.86 <sup>**#</sup>	126.32±21.98*	67*
LTG, ½ ED <sub>50</sub> +Digoxin	92.43±9.12 <sup>**</sup>	3.33±0.61 <sup>#</sup>	100	100 <sup>#</sup>	5.33±0.42	47.50±10.44 <sup>**##</sup>	155.99±1.88 <sup>**#</sup>	67*
<i>Strychnine-induced seizures</i>								
Control (untreated seizures)	7.73±0.46	1.33±0.21	100	100	6.00±0.00	1.15±0.64	8.88±0.76	100
Digoxin	31.28±9.17 <sup>**</sup>	0.67±0.21*	67*	33 <sup>**</sup>	3.00±1.10*	0.33±0.19	18.55±2.90	33 <sup>**</sup>
LTG, ED <sub>50</sub>	27.49±10.32*	1.50±0.62	67*	50 <sup>**</sup>	3.17±1.08 <sup>**</sup>	8.18±4.85	10.45	17 <sup>**</sup>
LTG, ½ ED <sub>50</sub>	26.12±10.77*	1.17±0.40	67*	50 <sup>**</sup>	2.83±0.98 <sup>**</sup>	2.79±2.01	9.98	17 <sup>**</sup>
LTG, ½ ED <sub>50</sub> +Digoxin	60.00±0.00 <sup>**#§°</sup>	0.00±0.00 <sup>**#§°</sup>	0 <sup>**#§§°</sup>	0 <sup>**#§§°</sup>	0.00±0.00 <sup>**#§°</sup>	0.00±0.00 <sup>**#§°</sup>	–	0 <sup>**#</sup>
<i>Camphor-induced seizures</i>								
Control (untreated seizures)	4.96±0.75	7.33±1.12	100	100	5.33±0.42	46.26±10.90	40.86±12.62	67
Digoxin	6.22±0.36	5.33±0.42	100	100	4.00±0.00*	44.85±7.42	–	0 <sup>**</sup>
LTG, ED <sub>50</sub>	8.08±2.35	4.67±0.67	100	100	5.00±0.45	39.61±9.34	49.85±19.26	50 <sup>##</sup>
LTG, ½ ED <sub>50</sub>	5.40±1.20	4.00±0.52*	100	100	5.00±0.45	21.42±5.16 <sup>#</sup>	27.84±9.59	50 <sup>##</sup>
LTG, ½ ED <sub>50</sub> +Digoxin	18.78±8.41 <sup>**°</sup>	3.50±1.18*	83	83	3.33±0.67 <sup>*§°</sup>	14.85±5.19 <sup>**#</sup>	25.46±9.82	50 <sup>##</sup>

Note:  $n$  – number of animals in each group; LTG – lamotrigine; \* –  $p < 0.05$  when compared with control; \*\* –  $p < 0.01$  when compared with control; # –  $p < 0.05$  when compared with digoxin, ## –  $p < 0.01$  when compared with digoxin; § –  $p < 0.05$  when compared with lamotrigine at an ED<sub>50</sub>; §§ –  $p < 0.01$  when compared with lamotrigine at an ED<sub>50</sub>; ° –  $p < 0.05$  when compared with lamotrigine at a ½ ED<sub>50</sub>; °° –  $p < 0.01$  when compared with lamotrigine at a ½ ED<sub>50</sub>.

The addition of the cardiac glycoside digoxin to the lamotrigine at a ½ ED<sub>50</sub> does not provide significant advantages in anticonvulsant action – the effect of the combination does not differ in its effectiveness from the action of AED at low dose, and according to the influence on some studied indicators of experimental paroxysms (in particular, the number of seizures per one mouse, % of animals with tonic convulsions, the duration of the convulsive period, etc.) is even significantly ( $p < 0.05$ ) inferior to digoxin monotherapy.

Comparable anticonvulsant efficacy of lamotrigine at both doses was verified in a strychnine-induced seizure model. Under the influence of AED, there was a statistically significant prolongation of the latent period of the first convulsions relative to the control, a decrease in the percentage of mice with clonic and tonic paroxysms, the severity of convulsions, and a significant reduction in animal lethality (17 % vs. 100 % in control,  $p < 0.01$ ).

Digoxin significantly increases the anticonvulsant potential of lamotrigine at a ½ ED<sub>50</sub>, significantly exceeding the effect of monotherapy with individual drugs – the combined use of cardiac glycoside with classical AED has the maximum protective effect, completely preventing the development of strychnine-induced seizures.

The effect of lamotrigine on the camphor-induced seizures is limited only by a statistically significant reduction in the number of clonic-tonic convulsion per one mouse under the influence of AED at a ½ ED<sub>50</sub> ( $p < 0.05$  relative to control) in the absence of significant effect of both conditionally effective and sub-effective doses of the drug on other studied parameters of experimental seizures.

The combination of lamotrigine at a ½ ED<sub>50</sub> and digoxin prolongs the onset of the first paroxysms (significantly relative to control, digoxin *per se*, and AED at a sub-effective dose,  $p < 0.05$ ), reduces the number of clonic-tonic convulsions per one mouse ( $p < 0.05$  relative to

the same group of animals with untreated convulsions), as well as statistically significantly reduces the severity of convulsions (relative to control and AED in both doses,  $p < 0.05$ ) and the duration of the convulsive period – relative to control groups and digoxin *per se*. However, in terms of the effect on the main integral indicator of efficiency – % lethality of animals – the combination does not differ significantly from the similar indicator of the control group, but also is statistically significantly inferior ( $p < 0.01$ ) to digoxin monotherapy.

## 5. Discussion

The different pathogenesis of experimental seizures makes it possible to assess the possible profile of the molecular effect of digoxin on epileptogenesis, as well as to further establish some neurochemical mechanisms of interaction of cardiac glycoside with known AEDs such as carbamazepine and lamotrigine.

The convulsive action of picrotoxin is associated with blockade of the chlorine ionophore GABA<sub>A</sub>-receptor and impaired GABA-ergic inhibitory neurotransmission [19]. The mechanism of convulsive action of thiosemicarbazide is the inhibition of the enzyme glutamate decarboxylase, which leads to the synchronous accumulation of the excitatory amino acid glutamate and depletion of the cerebral pool of GABA [20]. The convulsive effect of strychnine is due to blockade of postsynaptic glycine receptors [21]. Polymodal convulsive action of camphor is probably the result of impaired energy metabolism of neurons [22] and imbalance of cerebral monoamines [23].

Although the pronounced effect of sub-cardiotonic dose of digoxin on all four models of experimental seizures may indicate a complex mechanism of action of the drug on various neurochemical links in the development of paroxysms (such as increased GABA- and glycinergic neurotransmission, blockade of glutamate effects, restoration of tissue respiration of neurons, etc.), however, normalization of the transmembrane sodium current is likely – a key factor in maintaining ionic homeostasis of neurons, the violation of which is the final and main molecular predictor of seizure development. This effect is apparently achieved due to the effect on the neuronal Na<sup>+</sup>/K<sup>+</sup>-ATPase, an enzyme that transports sodium ions across excitable cell membranes [24, 25]. It has been shown that at low doses, cardiac glycosides activate (rather than inhibit) Na<sup>+</sup>/K<sup>+</sup>-ATPase, including neurons, thereby maintaining resting potential [26, 27].

The pronounced anticonvulsant potential of carbamazepine, primarily due to (but not limited to) the blockade of sodium channels in neuronal membranes [28], has been verified in most experimental convulsive models. This further confirms the feasibility of its widespread use in the treatment of various forms of epilepsy. Digoxin, however, significantly enhances the anticonvulsant effect of carbamazepine at low doses. In models of picrotoxin-, thiosemicarbazide- and camphor-induced seizures, the effectiveness of the combined use of carbamazepine and digoxin probably exceeds the effect not only of monotherapy with appropriate drugs, but also the

effect of AEDs in a higher, relatively effective dose. In the long term, this makes it possible to reduce the doses of classical AEDs (and, as a consequence, the risks of side effects) without losing the effectiveness of treatment. In addition, the clear modulation by digoxin of a moderate anticonvulsant effect of carbamazepine under conditions of camphor paroxysms indicates the possibility of the effect of such a combination on the course of refractory seizures.

Moderately narrow spectrum of anticonvulsant action of lamotrigine, presumably associated with limited influence of AED on certain neurochemical mechanisms of seizures (in particular, due to blockade of sodium channels, suppression of glutamatergic and, obviously, enhancing of glycinergic neurotransmission [29]) is confirmed by its pronounced effect on the model of thiosemicarbazide and strychnine convulsions in the absence of antagonism with picrotoxin and camphor. Digoxin somewhat enhances the effect of lamotrigine by the effect on individual studied indicators of the course of paroxysms induced by picrotoxin, thiosemicarbazide and camphor. However, even under such conditions, the combination is not able to significantly reduce lethality – the main integral indicator of the effectiveness of therapy. The most pronounced potentiating effect of digoxin was found only when combined with lamotrigine in the model of strychnine seizures, which provides the maximum protective effect – complete prevention of seizures.

Thus, digoxin has its own polymodal anticonvulsant effect, which is constantly manifested in models of paroxysms with different neurochemical mechanisms. In addition, digoxin has potentiating properties: cardiac glycoside significantly enhances the anticonvulsant effect of carbamazepine and moderately lamotrigine, which provides a protective effect of sub-effective doses of classical AEDs, including models with non-profile for drugs mechanisms of action. In the long run, this will not only reduce the dose of AEDs and, consequently, the risks of side effects without losing the effectiveness of treatment, but also the ability to affect resistant seizures.

**Study limitations.** The influence of digoxin on the spectrum of anticonvulsant action of another widespread antiepileptic drugs (such as sodium valproate, levetiracetam, topiramate, clonazepam and phenobarbital) remains unclear.

**Prospects for further research.** Elucidation of the effect of digoxin on the spectrum of anticonvulsant activity of sodium valproate, levetiracetam, topiramate, clonazepam and phenobarbital as well as determination of neurochemical mechanisms of anticonvulsant action of digoxin – both in monotherapy and in combinations with well-known antiepileptic drugs.

## 6. Conclusion

The effect of digoxin in sub-cardiotonic dose on the anticonvulsant potential of carbamazepine and lamotrigine under conditions of picrotoxin-, thiosemicarbazide-, strychnine- and camphor-induced seizures was studied. It was found that digoxin not only has its own

permanent anticonvulsant effect on different models of paroxysms with different neurochemical mechanisms of development, but also significantly enhances the anticonvulsant potential of carbamazepine (to a lesser extent – lamotrigine) regardless of the pathogenesis of experimental paroxysms. Based on the results, it can be concluded that digoxin has a high potential as an adjuvant medicine in complex epilepsy treatment because it enhances the efficiency of low-dose traditional anticonvulsants carbamazepine and lamotrigine.

#### Conflict of interests

The authors declare that they have no conflicts of interest.

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