Original Article

Thai Journal of Pharmaceutical Sciences

Digoxin potentiates the anticonvulsant effect of carbamazepine and lamotrigine against experimental seizures in mice

Vadim Tsyvunin[1], Sergiy Shtrygol[1], Diana Shtrygol[2], Mariia Mishchenko[1], Ihor Kapelka[1], Andriy Taran[1]

[1]Department of Pharmacology and Pharmacotherapy, National University of Pharmacy, Kharkiv, Ukraine, [2]Department of Clinical Neurology, Psychiatry, and Narcology, School of Medicine, V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

Corresponding Author:
Dr. Vadim Tsyvunin,
Department of Pharmacology and Pharmacotherapy,
National University of Pharmacy, Kharkiv - 61002, Ukraine.
Phone: +38 096 8565831,
Fax: +38 057 7063069.
E-mail: tsyvunin-vad@ukr.net

Received: Nov 11, 2020
Accepted: Dec 09, 2020
Published: Jun 30, 2021

ABSTRACT

Introduction: The worldwide high amount of multidrug-resistant epilepsy patients makes it urgent to find new approaches to treating, including the use of combinations of classic anticonvulsants with drugs that have an exclusively original mechanism of action, in particular digoxin. **Objective:** The aim of this work was to investigate the influence of low-dose cardiac glycoside digoxin on the anticonvulsant effect of carbamazepine and lamotrigine. **Methods:** Basic models of pentylenetetrazole-induced seizures and electroinduced paroxysms (maximal electroshock) in mice were used. Classic anticonvulsants were administered intragastrically in conditionally effective and sub-effective doses at 30 min, digoxin – subcutaneously at a dose of 0.8 mg/kg (1/10 LD50) at 15 min before seizures induction. Pentylenetetrazole at a dose of 80 mg/kg was administered subcutaneously. The maximal electroshock was reproduced by transmitting an electric current (strength – 50 mA, frequency – 50 Hz) through the corneal electrodes for 0.2 sec. **Results:** It was established that the co-administration of digoxin with carbamazepine and lamotrigine in sub-effective doses render a marked anticonvulsant action on the model of pentylenetetrazole-induced seizures as well as on seizures induced by maximal electroshock. Digoxin can reduce the doses of carbamazepine and lamotrigine with a corresponding reduction in the risk of side effects without compromising treatment efficacy. **Conclusion:** Digoxin can be a valuable component of complex therapy of epilepsy

Keywords: Carbamazepine, combined therapy, digoxin, experiment, lamotrigine, seizures

INTRODUCTION

The great amount of epilepsy patients in the world population, a large percentage of multidrug-resistant forms, especially in children up to 30%,[1-3] determines the urgency of developing new approaches to treatment, in particular, the use in basic treatment regimens the drugs, which have mechanisms of action not known for their antiepileptic action and are able to overcome pharmacoresistance along with classical anticonvulsants.

Given a certain similarity in the pathogenesis of epilepsy and arrhythmia, in particular, dysregulation of ion fluxes, activation of the voltage-gated membrane channels, increased excitability, and the relationship between anticonvulsant and antiarrhythmic action were suggested,[4] which was confirmed experimentally and clinically. It has been established that certain antiarrhythmic drugs – sodium channel blockers lidocaine and propafenone, β-adrenoblockers propranolol, metoprolol, pindolol, potassium channel blocker amiodarone, calcium channel blockers nifedipine, amlodipine, cinnarizine, diltiazem, and verapamil additionally have anticonvulsant properties.[5-8] In addition, the ability of some modulators of ionic currents (in particular, non-selective blocker of sodium channels mexiletine and sinus node I_f-channels blocker ivabradine) to enhance the anticonvulsant effect of classical antiepileptic drugs has been verified.[9-11] Improved treatment of epilepsy with the addition of cardiac glycoside digoxin in subcardiotonic doses to classical anticonvulsants has been shown. From separate early studies, there is evidence of

a pronounced anticonvulsant effect of digoxin in models of seizures induced by pentylenetetrazole, bemegride, thiosemicarbazide, strychnine, and maximal electroshock [MES] in mice and rats, seizure focus in hippocampus of frogs and rats, molluscan pacemaker neurons.[12,13] However, the optimal combinations of classical anticonvulsants and digoxin in certain forms of epilepsy, dosage regimen, mechanisms of interaction, as well as new targets of pharmacotherapeutic influence on convulsive syndrome remain unknown.

Previously, we found that digoxin at a dose of $^1/_{10}$ LD_{50} enhances the anticonvulsant activity of sodium valproate, topiramate, levetiracetam, phenobarbital, and clonazepam in the model of pentylenetetrazole-induced seizures.[14]

The aim of the present study is investigating the influence of low doses of cardiac glycoside digoxin on the anticonvulsant effect of carbamazepine and lamotrigine under conditions of pentylenetetrazole- and electro-induced seizures.

MATERIALS AND METHODS

Animals and Treatment

The experiments were conducted on 112 random-bred male albino mice weighing 18–22 g in accordance with the principles and requirements of the EU Council Directive (2010) on the protection of animals used for scientific purposes. Mice were kept on a standard diet of vivarium with free access to water, constant humidity, and temperature +18–20°C based on the Central Research Laboratory of the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy (Kharkiv, Ukraine).

The anticonvulsant properties of combinations of carbamazepine and lamotrigine with digoxin have been studied in basic models of seizures with different pathogenesis caused by pentylenetetrazole and MES.[15,16] Animals were randomly divided into groups: Group 1 – control (untreated seizures), Group 2 – animals with model seizures receiving digoxin, and the remaining groups – animals with seizures, which were administered carbamazepine, lamotrigine, and their combinations with digoxin.

Classical anticonvulsants were administered once intragastrically (i.g.) in the form of an aqueous suspension stabilized with Tween-80, in conditionally effective (ED_{50}) and sub-effective (½ ED_{50}) doses 30 min before seizure simulation: Carbamazepine (Finlepsin, Teva Operations Poland, Poland) at doses of 100 and 50 mg/kg and lamotrigine (Lamictal, GlaxoSmithKline Pharmaceuticals, Poland) – at doses of 25 and 12.5 mg/kg.[17] Digoxin (DNCLZ/Health, Ukraine) was administered once subcutaneously at a dose of 0.8 mg/kg ($^1/_{10}$ LD_{50})[12,13] for 15 min before induction by seizures. Control animals received i.g. purified water in appropriate volume (0.1 ml/10 g of body weight).

Pentylenetetrazole-induced Seizures

Pentylenetetrazole (Sigma, USA) as an aqueous solution at a dose of 80 mg/kg was administered subcutaneously to animals. Immediately after the introduction of the convulsant, the animals were placed into the separate transparent plastic cylindrical containers and continuously monitored for 60 min.[15,16] The effectiveness of anticonvulsant drugs and their combinations was evaluated by the following indicators: Latency period of first convulsions (latency), the number of clonic-tonic seizures in 1 mouse, percentage of animals in the group separately with clonic and tonic convulsions, severity of seizure – in points (1 point – single tremors, 2 points – "manege" running or "kangaroo" position, 3 points – clonic convulsions without lateral position, 4 points – clonic-tonic convulsions with lateral position, 5 points – tonic extension, and 6 points – tonic extension, which led to the death of the animal), duration of convulsive period (period of seizures), life expectancy of animals to death (time to death), and lethality. If seizures were not observed for 1 h, the latency was considered to be 60 min.[18]

MES

MES was reproduced by passing an electric current with constant characteristics (power – 50 mA, frequency – 50 Hz) through copper corneal electrodes for 0.2 s. The activity of anticonvulsant drugs and their combinations was evaluated by the following indicators: Percentage of animals in the group separately with clonic and tonic convulsions, seizure severity in points (3 points – clonic seizures without lateral position, 4 points – clonic-tonic seizures with lateral position, 5 points – tonic extension, and 6 points – tonic extension, which led to the death of the animal), the duration of the convulsive period (period of seizures), the time of recovery from the lateral position (restoration of motor activity), life expectancy of animals to death (time to death), and lethality.[15,16]

Statistical Analysis

For statistical analysis, STATISTICA 8.0 for Windows was used. The results are expressed as mean ± standard error of mean. The level of statistical significance was considered as $P < 0.05$. Statistical differences between groups were analyzed using the parametric Student's *t*-test – in cases of normal distribution, non-parametric Mann–Whitney U-test – in its absence. For the results in the alternative form (lethality, percentage of mice with clonic and tonic convulsions), the Fisher's angular transformation (with Yates correction, if necessary) was used.

RESULTS

In the model of pentylenetetrazole seizures in mice, digoxin at a dose of 0.8 mg/kg shows moderate anticonvulsant properties [Tables 1 and 2], which are verified by the significant prolongation of the latency, as well as increasing the time to death relative to control (in 2.7 and 3.1 times, respectively, $P < 0.01$). In addition, digoxin reduces the lethality of mice by almost a quarter compared to the group of animals with untreated seizures, although this difference is tendential ($P > 0.05$).

Carbamazepine in both studied doses (100 and 50 mg/kg) in the model of pentylenetetrazole-induced seizures [Table 1] had almost no anticonvulsant effect. Only at large dose, the drug caused a statistically significant increase in the duration of period of seizures and, accordingly, the lifetime of animals to death (5.7 and 5.4 times, respectively) compared to control.

It should be noted, however, that against the background of carbamazepine at an ED_{50} convulsive period was quite severe,

Table 1: Anticonvulsant effect of digoxin, carbamazepine, and their combination in the pentylenetetrazole-induced seizures in mice, mean ± standard error of mean

Group of animals	*n*	Dose, mg/kg	Latency, min	Number of clonic-tonic seizures in 1 mouse	Percentage of mice with convulsions		Severity of seizures, points	Period of seizures, min	Time to death, min	Lethality, %
					Clonic	Tonic				
Control	10	–	4.18±0.63	3.10±0.72	100	100	5.80±0.20	5.39±2.34	7.26±1.21	90
Digoxin	6	0.8	11.18±1.81**	3.83±0.65	100	83	5.17±0.54	12.46±2.58	22.28±1.30**	67
Carbamazepine	6	100	3.73±0.50$^{\#\#}$	1.67±0.21$^{\#}$	100	100	5.67±0.33	30.77±9.50**	40.56±9.55**	83
Carbamazepine	6	50	5.75±0.92$^{\#}$	1.17±0.17$^{\#\#}$	100	100	5.33±0.42	13.63±11.21	27.32±16.26	67
Carbamazepine+Digoxin	6	50+0.8	19.87±8.39*	2.00±0.58	83	83	4.00±0.89*	21.61±10.61	45.42±24.92**	33**§

n: Number of animals, *P<0.05 when compared with control, **P<0.01 when compared with control, $^{\#}P$<0.05 when compared with digoxin, $^{\#\#}$<0.01 when compared with digoxin, $^{\S}P$<0.05 when compared with carbamazepine at an ED_{50} (100 mg/kg)

Table 2: Anticonvulsant effect of digoxin, lamotrigine, and their combination in the pentylenetetrazole-induced seizures in mice, mean ± standard error of mean

Group of animals	*n*	Dose, mg/kg	Latency, min	Number of clonic-tonic seizures in 1 mouse	Percentage of mice with convulsions		Severity of seizures, points	Period of seizures, min	Time to death, min	Lethality, %
					Clonic	Tonic				
Control	10	–	4.18±0.63	3.10±0.72	100	100	5.80±0.20	5.39±2.34	7.26±1.21	90
Digoxin	6	0.8	11.18±1.81**	3.83±0.65	100	83	5.17±0.54	12.46±2.58	22.28±1.30**	67
Lamotrigine	6	25	4.17±0.93$^{\#\#}$	1.50±0.22$^{\#\#}$	100	100	6.00	7.92±1.68	12.09±0.53*$^{\#\#}$	100$^{\#}$
Lamotrigine	6	12.5	3.01±0.45$^{\#\#}$	2.33±0.33	100	100	6.00	7.37±2.01	10.38±2.18$^{\#\#}$	100$^{\#}$
Lamotrigine+Digoxin	6	12.5+0.8	35.89±10.90**$^{\#\S\circ}$	1.00±0.63*$^{\#}$	50**$^{\#\#\S\S\circ\circ}$	50**$^{\S\S\circ\circ}$	3.00±1.34*$^{\S\circ}$	4.81±3.91	21.41±6.54**$^{\circ}$	50*$^{\S\S\circ\circ}$

n: Number of animals, *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, $^{\S}P$<0.05 when compared with lamotrigine at an ED_{50} (25 mg/kg), $^{\S\S}P$<0.01 when compared with lamotrigine at an ED_{50} (25 mg/kg), $^{\circ}P$<0.05 when compared with lamotrigine at a ½ ED_{50} (12.5 mg/kg), $^{\circ\circ}P$<0.01 when compared with lamotrigine at a ½ ED_{50} (12.5 mg/kg)

as indicated by clinical signs – lack of motor activity of animals with prolonged lateral position (without tonic extension), as well as deep irregular breathing mice by gasping type.

The combined use of digoxin and carbamazepine at a low dose (½ ED_{50}) has a pronounced anticonvulsant effect. Against this background, there is not only a statistically significant reduction in the lethality of mice in the group by 57% relative to control but also a probable increase in the latency period of the first attacks and the lifetime of animals to death by 4.6 and 6.3 times, respectively. In addition, the combination of digoxin with carbamazepine at a sub-effective dose by 45% ($P < 0.05$) reduces the severity of seizures compared to a similar indicator in the group of control animals.

Lamotrigine, like carbamazepine, also did not show a pronounced anticonvulsant effect in the model of pentylenetetrazole-induced seizures [Table 2]. In both the effective (ED_{50}) and sub-effective (½ ED_{50}) doses, lamotrigine did not prevent animal lethality, did not affect the latency, percentage of mice with clonic and tonic seizures, severity of seizures, and period of seizures. The influence of lamotrigine at the effective dose (25 mg/kg) on the course of pentylenetetrazole-induced seizures was limited only by a statistically significant increase in the lifetime to death by 1.7 times and a tendency to reduce the number of clonic-tonic seizures by 2 times compared with control.

The combination of lamotrigine at a sub-effective dose (½ ED_{50}) and digoxin exhibits potent anticonvulsant properties, as determined by the main integral indicator of efficacy – a significant reduction lethality in group – not only compared to control but also compared to groups of animals receiving lamotrigine at the doses of 25 and 12.5 mg/kg. The pronounced anticonvulsant effect of the combination of lamotrigine with digoxin is further confirmed by statistically significant influence on all other markers of experimental seizures. Thus, lamotrigine at a sub-effective dose of 12.5 mg/kg with digoxin is significantly not only compared to control but also compared to groups of animals treated with both digoxin and lamotrigine in high and low doses, prolongs the latency (8.6, 3.2, 8.6, and 11.9 times, accordingly), and reduces the percentage of mice with clonic seizures by half. In addition, against the background of the combination, there is a 2-fold statistically significant decrease in the percentage of animals with tonic convulsions and the severity of seizures compared to the control and lamotrigine groups in both ED_{50} and ½ ED_{50}. Lamotrigine in combination with digoxin also reduces the number of clonic-tonic seizures in 1 mouse in 3.1 and 3.8 times compared to the same indicator in the control and digoxin groups, respectively ($P < 0.05$), and significantly increases the lifetime of animals to death in 2.9 times compared to the control and 2.1 times compared to the group of lamotrigine at the effective dose.

In the model of seizures induced by MES [Tables 3 and 4], digoxin exhibits pronounced anticonvulsant properties: Reduces lethality by 7 times compared to control, decreases the severity of seizures by 24% and also the duration of the convulsive period by 77% ($P < 0.05$), and also significantly prolongs the lifetime of animals to death.

The effects of carbamazepine and its combination with digoxin on the indicators of electro-induced seizures are

Table 3: Anticonvulsant effect of digoxin, carbamazepine, and their combination in the maximal electroshock seizure test in mice, mean ± standard error of mean

Group of animals	*n*	Dose, mg/kg	Percentage of mice with convulsions		Severity of seizures, points	Period of seizures, s	Recovery period/ lateral position, s	Time to death, s	Lethality, %
			Clonic	Tonic					
Control	8	–	100	100	5.88±0.12	14.13±0.85	18.00	13.57±0.75	88
Digoxin	8	0.8	100	100	4.75±0.25**	8.00±2.53*	21.43±9.82	19.00*	13**
Carbamazepine	8	100	88	25**###	2.88±0.44**###	1.25±0.16**###	8.57±1.66**	–	0**
Carbamazepine	7	50	100	43**###	3.43±0.20**###	1.57±0.20**###	9.00±2.54**	–	0**
Carbamazepine+Digoxin	8	50+0.8	75*#°	0**###§°°	2.25±0.49**##°	1.13±0.13**###	6.17±0.60**###	–	0**

n: Number of animals, *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 carbamazepine at an ED_{50} (100 mg/kg), °P<0.05 when compared with carbamazepine at a ½ ED_{50} (50 mg/kg), °°P<0.01 when compared with carbamazepine at a ½ ED_{50} (50 mg/kg)

Table 4: Anticonvulsant effect of digoxin, lamotrigine, and their combination in the maximal electroshock seizure test in mice, mean ± standard error of mean

Group of animals	*n*	Dose, mg/kg	Percentage of mice with convulsions		Severity of seizures, points	Period of seizures, s	Recovery period/ lateral position, s	Time to death, s	Lethality, %
			Clonic	Tonic					
Control	8	–	100	100	5.88±0.12	14.13±0.85	18.00	13.57±0.75	88
Digoxin	8	0.8	100	100	4.75±0.25**	8.00±2.53*	21.43±9.82	19.00*	13**
Lamotrigine	7	25	100	43**##°	3.43±0.20**##	1.14±0.14**##	22.43±8.97	–	0**
Lamotrigine	7	12.5	100	86§	3.86±0.14**#	1.71±0.18**##	11.00±1.59**	–	0**
Lamotrigine+Digoxin	7	12.5+0.8	100	43**##°	3.43±0.20**##	1.14±0.14**##	9.57±2.50**	–	0**

n: Number of animals, *$P<0.05$ 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_{50} (25 mg/kg), °$P<0.05$ when compared with lamotrigine at a ½ ED_{50} (12.5 mg/kg)

given in Table 3. In both doses ED_{50} (100 mg/kg) and ½ ED_{50} (50 mg/kg), the drug shows a pronounced anticonvulsant effect: Completely prevents lethality of animals in experimental groups, statistically significant compared to control and digoxin reduces the % of mice with tonic convulsions, severity of seizures, duration of the convulsive period, and the recovery period from the lateral position. However, the combination of low-dose carbamazepine and digoxin has much more pronounced anticonvulsant properties: In addition to complete protection against death, the combination completely prevents the development of tonic seizures, and by a quarter compared to control groups, digoxin and low-dose carbamazepine decrease the percentage of mice with clonic convulsions ($P < 0.05$). In addition, carbamazepine in combination with digoxin statistically significantly reduces the severity of seizures (2.6, 2.1, and 1.5 times compared to similar indicator in the control groups, monotherapy digoxin, and carbamazepine in low doses, respectively), significantly compared to control and digoxin reduces the period of seizures and the recovery time from the lateral position.

The results of the study of the effect of lamotrigine and its combination with digoxin on the course of paroxysms induced by MES are given in Table 4.

Like carbamazepine, lamotrigine in both ED_{50} (25 mg/kg) and ½ ED_{50} (12.5 mg/kg) exhibits pronounced anticonvulsant properties: Completely prevents animal lethality, statistically significant relative to control and digoxin reduces the severity and the period of seizures. At an ED_{50}, lamotrigine is additionally more than 2-fold compared to control and digoxin, as well as exactly twice against the same indicator on the background of ½ ED_{50} reduces the percentage of mice with tonic convulsions. In addition, at a dose of 12.5 mg/kg, lamotrigine significantly reduces the time of recovery from the lateral position compared to the control. The combined use of lamotrigine at a dose of 12.5 mg/kg and digoxin is characterized by an even more pronounced anticonvulsant effect than monotherapy with each drug. Thus, the combination, along with 100% protection of animals from death as well as lamotrigine in ED_{50}, reduces the percentage of mice with tonic convulsions, similarly reducing the severity of paroxysms and the period of seizures compared to the control and digoxin groups. Finally, the combined use of lamotrigine and digoxin reduces the recovery period of animals from the lateral position (1.9 times compared to control, $P < 0.05$).

DISCUSSION

To assess the effect of digoxin on the anticonvulsant effect of carbamazepine and lamotrigine, seizure models induced by pentylenetetrazole and MES were selected. This is due to qualitative differences in their pathogenesis and the manifestation of seizures.[16] Pentylenetetrazole is a classic antagonist of the inhibitory mediator GABA, blocker of GABA site of GABA-barbiturate-benzodiazepine receptor complex. In the model of pentylenetetrazole seizures, the most pronounced anticonvulsant effect is shown by antiepileptic drugs with GABAergic properties – benzodiazepines, barbiturates, valproates, vigabatrin, and tiagabine.[19,20] The MES model is based on the induction of sodium currents,

membrane hyperpolarization, and excitation generalization. Under the conditions of electro-induced seizures, the blockers of voltage-gated sodium channels (phenytoin, carbamazepine, oxcarbazepine, and lamotrigine) show pronounced anticonvulsant properties.[19,20]

The lack of anticonvulsant effect of carbamazepine and lamotrigine in the model of pentylenetetrazole-induced seizures, along with the clear effect of each drug on the course of electro-induced paroxysms, is in good agreement with the known data on the predominant mechanism of action of drugs.[19,20] In addition, under conditions of pentylenetetrazole-induced seizures, our results confirm the data on the limited spectrum of anticonvulsant action of carbamazepine, the use of which, in particular, is ineffective in myoclonic epilepsy. It is known that carbamazepine is able to exacerbate myoclonic seizures,[20] which correlates with a more severe course of pentylenetetrazole-induced seizures on the background of the drug in our experiments.

Digoxin, in turn, has an antagonistic effect with pentylenetetrazole, which indirectly indicates the presence of GABAergic properties. However, a more pronounced anticonvulsant effect of digoxin was verified under the conditions of electro-induced seizures in the MES test. There is evidence that digoxin has a dose-dependent (sub-cardiotonic dose activates, cardiotonic inhibits) activity of Na^+, K^+-ATPase not only of cardiomyocyte membranes but also neurons, which provides the basic mechanism of excitation and conduction.[21,22] This mechanism is not inherent in existing antiepileptic drugs, which makes it promising to continue studying digoxin as an anticonvulsant in the adjuvant therapy of epilepsy, including multidrug-resistant one.

The combined use of digoxin with carbamazepine and lamotrigine in sub-effective doses drastically changes the effect of the classical anticonvulsants on the pentylenetetrazole-induced seizures model. The combinations have a powerful anticonvulsant effect, while monotherapy with carbamazepine and lamotrigine, even in conditionally effective doses, is generally ineffective.

The potential found in the anticonvulsant action of carbamazepine and lamotrigine by digoxin was verified by the MES test. Although under conditions of electro-induced seizures classic anticonvulsants even at low doses show a pronounced protective effect, digoxin can further enhance the anticonvulsant properties of drugs. The combined use of lamotrigine at a sub-effective dose and digoxin shows an anticonvulsant effect at the level of lamotrigine in ED_{50}. The combination of digoxin with carbamazepine at a low dose is not only inferior to the severity of the anticonvulsant effect of monotherapy with a classic anticonvulsant in a high dose but also probably exceeds it, in particular, by the complete reduction of the tonic component of seizures.

Therefore, in basic models of seizures (pentylenetetrazole induced and MES) in mice, it was found that digoxin at a sub-cardiotonic dose of 0.8 mg/kg significantly enhances the effect of classical antiepileptic drugs carbamazepine and lamotrigine, providing a clear protective effect on their sub-effective doses. This suggests that digoxin may be a valuable component of complex pharmacotherapy of epilepsy, as it allows reducing the dose of classical anticonvulsants with a corresponding reduction in the risk of side effects without compromising the effectiveness of treatment. Despite the fact that digoxin belongs to drugs with narrow therapeutic index, it should be emphasized that low (sub-cardiotonic) doses of digoxin are well tolerated and cardiac signs of possible toxic effects (bradycardia, cardiac conduction disturbances, etc.) are absent, as shown previously.[13] Features of pharmacodynamics of digoxin – abrupt development of cardiac effects with increasing dose and, consequently, the lack of impact on the myocardium of low doses of digoxin – are enough to predict the safety of its use as an anticonvulsant.

Along with the pharmacodynamic interaction of digoxin with classical anticonvulsants, leading to an increase in the anticonvulsant action, the role of possible pharmacokinetic interaction cannot be excluded. The pharmacokinetic interaction of several antiepileptic drugs with digoxin is known. For example, phenytoin, carbamazepine, and topiramate can decrease plasma concentrations of digoxin.[23-25] To clarify the possibility of changing the concentration of antiepileptic drugs in the blood and, especially, in the brain under the influence of digoxin, special studies are needed, which are to be assigned to the next stage of our study. Subsequent analysis of ultrastructural changes in the brain under monotherapy with antiepileptic drugs and their combination with digoxin will also contribute to the clarification of the subtle mechanisms of modulation of the specific action of anticonvulsants by digoxin.

Thus, the results of the research indicate the topicality of in-depth study of the influence of digoxin on the anticonvulsant effect of classical antiepileptic drugs: Study of interaction with other anticonvulsants, spectrum, and mechanisms of anticonvulsant action in models of seizures with different pathogenesis. The results also substantiate the prospects for improving the treatment of epilepsy (including multidrug resistant) through the combined use of classical anticonvulsants with other drugs.

CONCLUSION

The effect of low-dose cardiac glycoside digoxin on the anticonvulsant effect of carbamazepine and lamotrigine under conditions of pentylenetetrazole-induced seizures and MES test has been studied. Coadministration of digoxin with carbamazepine and lamotrigine in sub-effective doses shows pronounced anticonvulsant properties in the model of pentylenetetrazole-induced seizures in the absence of the effect of classical anticonvulsants in both sub-effective and conditionally effective doses. Digoxin enhances the action of carbamazepine and lamotrigine, providing a clear protective effect of their sub-effective doses on the model of seizures induced by MES. Digoxin can be a valuable component of complex therapy of epilepsy, as it enables to reduce the dose of classic anticonvulsants carbamazepine and lamotrigine with a corresponding reduction in the risk of side effects without compromising the effectiveness of treatment.

AUTHORS' CONTRIBUTIONS

VT and SS conceived and designed the research. VT, DS, MM, IK, and AT conducted experiments. VT and SS analyzed

data. VT and DS wrote the manuscript. All authors read and approved the manuscript.

FUNDING

The research was conducted as a part of a fundamental scientific study of the Ministry of Health of Ukraine "Rationale for improving the treatment of multidrug-resistant epilepsy through the combined use of classical anticonvulsant medicines with other drugs" (No. 0120U102460, 2020/2022), carried out at the expense of the State Budget of Ukraine.

REFERENCES

1. Abramovici S, Bagić A. Epidemiology of epilepsy. Handb Clin Neurol 2016;138:159-71.
2. Kalilani L, Sun X, Pelgrims B, Noack-Rink M, Villanueva V. The epidemiology of drug-resistant epilepsy: A systematic review and meta-analysis. Epilepsia 2018;59:2179-93.
3. Scheffer I, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, *et al*. ILAE classification of the epilepsies: Position paper of the ILAE commission for classification and terminology. Epilepsia 2017;58:512-21.
4. Borowicz K, Banach M. Antiarrhythmic drugs and epilepsy. Pharmacol Rep 2014;66:545-51.
5. De Sarro G, Di Paola E, Ferreri G, De Sarro A, Fischer W. Influence of some β-adrenoceptor antagonists on the anticonvulsant potency of antiepileptic drugs against audiogenic seizures in DBA/2 mice. Eur J Pharmacol 2002;442:205-13.
6. El-Azab M, Moustafa Y. Influence of calcium channel blockers on anticonvulsant and antinociceptive activities of valproic acid in pentylenetetrazole-kindled mice. Pharmacol Rep 2012;64:305-14.
7. Larkin J, Besag F, Cox A, Williams J, Brodie MJ. Nifedipine for epilepsy? A double-blind, placebo-controlled trial. Epilepsia 1992;33:346-52.
8. Zeiler F, Zeiler K, Kazina C, Teitelbaum J, Gillman LM, West M. Lidocaine for status epilepticus in adults. Seizure 2015;31:41-8.
9. Sawicka K, Załuska K, Wawryniuk A, Załuska-Patel K, Szczyrek M, Drop B, *et al*. Ivabradine attenuates the anticonvulsant potency of lamotrigine, but not that of lacosamide, pregabalin and topiramate in the tonic-clonic seizure model in mice. Epilepsy Res 2017;133:67-70.
10. Sawicka K, Wawryniuk A, Zwolak A, Daniluk J, Szpringer M, Florek-Luszczki M, *et al*. Influence of ivabradine on the anticonvulsant action of four classical antiepileptic drugs against maximal electroshock-induced seizures in mice. Neurochem Res 2017;42:1038-43.
11. Wróblewska D, Rudkowska M, Banach M, Borowicz-Reutt KK. Interactions of mexiletine with novel antiepileptic drugs in the maximal electroshock test in mice: An isobolographic analysis. Neurochem Res 2018;43:1887-96.
12. Markova IV, Mikhaĭlov IB, Guzeva VI. Digoxin an active antiepileptic agent. Pharmacol Toxicol 1991;54:52-4.
13. Shtrygol SY, Shtrygol DV. Digoxin as an antiepileptic in children (clinical and experimental study). Ukrain Med Almanac 2010;13:164.
14. Tsyvunin V, Shtrygol S, Shtrygol D. Digoxin enhances the effect of antiepileptic drugs with different mechanism of action in the pentylenetetrazole-induced seizures in mice. Epilep Res 2020;167:106465.
15. Hock FJ. Drug Discovery and Evaluation: Pharmacological Assays. Berlin, Germany: Springer International Publishing; 2016.
16. Mironov AN, Bunyatyan ND, Vasileva AN, editors. Guidelines for Conducting Pre-clinical Trials of Medicines. Moscow: Grif and K; 2012.
17. Duveau V, Pouyatos B, Bressand K, Bouyssières C, Chabrol T, Roche Y, *et al*. Differential effects of antiepileptic drugs on focal seizures in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. CNS Neurosci Ther 2016;22:497-506.
18. Tsyvunin V, Shtrygol' S, Gorbatch T, Shtrygol D. Anticonvulsant activity of *Fumaria schleicheri* dry extract and sodium valproate: Role of neurotrophin and cytokine pathways. Thai J Pharm Sci 2020;44:46-51.
19. Löscher W. The holy grail of epilepsy prevention: Preclinical approaches to antiepileptogenic treatments. Neuropharmacology 2020;167:107605.
20. Waller D, Sampson A. Medical Pharmacology and Therapeutics. 5th ed. Amsterdam, Netherlands: Elsevier; 2018.
21. De Lores Arnaiz GR, Ordieres MG. Brain Na^+, K^+-ATPase activity in aging and disease. Int J Biomed Sci 2014;10:85-102.
22. Sergeev PV, Shimanovskii NL. Biochemical Pharmacology. Moscow: OOO Meditsynskoe Informatsionnoe Agenstvo; 2010.
23. Johannessen SI, Landmark CJ. Antiepileptic drug interactions principles and clinical implications. Curr Neuropharmacol 2010;8:254-67.
24. Patsalos PN, Fröscher W, Pisani F, van Rijn CM. The importance of drug interactions in epilepsy therapy. Epilepsia 2002;43:365-85.
25. Perucca E. Clinically relevant drug interactions with antiepileptic drugs. Br J Clin Pharmacol 2006;61:246-55.