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Digoxin potentiates the anticonvulsant effect of low-dose phenobarbital and clonazepam under primary generalized seizures with different neurochemical mechanisms

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Epilepsy is a chronic brain disease of non-infectious origin with periodic seizures as the main symptom. Epilepsy is capable of onset at any age, although a third of all patients are children under 12 years [1]. The disease is the most common psychoneurological pathological condition in the world. According to the World Health Organization, there are more than 50 million people suffering from epilepsy worldwide [2]. The vast majority of patients (about 80 %) live in low- and middle-income countries (including Ukraine). At the same time, more than half of patients with epilepsy do not have access to proper diagnosis and treatment [2]. The last few decades, in addition, there is an unfavorable trend towards the spread of multidrug-resistant epilepsy characterized by the resistance to all existing antiepileptic drugs [1, 3]. According to statistics, one in four adult patients suffers from resistant (or refractory) epilepsy, and among children this amount reaches almost 30 % [4, 5]. Epilepsy not only negatively affects the quality of life (in particular, it limits the ability of patients to work, to drive vehicles as well as clearly increases the risk of social stigma) [6, 7]. Moreover, it is likely to be found that epilepsy, even with adequate treatment, almost triples the risk of premature death in patients compared with the population average [4, 8]. That is why it is important to find and develop new – more effective and safer – antiepileptic drugs that can not only control the disease, prevent seizures, but also improve the quality and life expectancy of patients with epilepsy.

Specific antiepileptic drugs (AEDs) are used to prevent or correct convulsive states in epilepsy [9, 10]. However, existing AEDs have limited influence on epileptic paroxysms' pathogenesis (such as blocking voltage-gated sodium channels of neurons, enhancing the inhibitory effect of cerebral GABA, blocking glutamate receptors, etc.), which limits their use to certain clinical types of seizures [9, 11, 12]. In addition, every fourth patient with epilepsy is multidrug-resistant – completely insensitive to all AEDs, including modern ones [8, 13].

One of the ways to overcome the pharmacoresistance of epilepsy is the use of subcardiotonic doses of the cardiac glycoside digoxin, which has been proven experimentally [14] and requires further research. Obviously, this positive effect is associated with the impact on the activity of Na^+, K^+-ATPase, i. e. interference with the fundamental mechanisms of maintaining the membrane potential and, accordingly, the

excitability of neurons [15, 16]. This mechanism is not inherent in any of the available AEDs.

In previous studies, we found a clear potentiation of digoxin at the dose, which do not affect the myocardium, the effect of a number of AEDs at subeffective doses under the basic screening model of seizures induced by pentylenetetrazole [17]. It was found that digoxin also significantly enhances the anticonvulsant potential of carbamazepine (to a lesser extent – lamotrigine) under condition of chemo-induced seizures with different pathogenesis [18]. However, the neurochemical mechanisms of anticonvulsant action of digoxin and its combinations with classical commonly used AEDs with known GABA-ergic properties – phenobarbital and clonazepam – remain unclear.

The aim of this study – to investigate the influence of cardiac glycoside digoxin on the anticonvulsant activity of low-dose phenobarbital and clonazepam under models of primary generalized seizures with different neurochemical mechanisms.

Material and methods. The research leading to these results has received funding from the Ministry of Health of Ukraine, under the project number 0120U102460 «Rationale for improving the treatment of multidrug-resistant epilepsy through the combined use of classical anticonvulsants with other drugs». A total of 192 random-bred male albino mice (age – 3–4 months) weighing 22–25 g have been used. Animals were kept under controlled vivarium conditions at constant humidity and temperature + 18–20 °C on a standard diet with free access to water on the Central Scientific and Research Laboratory of the Educational and Scientific Institute of Applied Pharmacy of the National University of Pharmacy – ESIAP (Kharkiv, Ukraine). The experiments were carried out in accordance with bioethical principles and standards of the EU Directive 2010/63/EU (2010). All the experimental protocols have been approved by the Bioethics Commission of the National University of Pharmacy (protocol No. 3, September 10, 2020).

The anticonvulsant effect of digoxin and its combinations with AEDs has been studied on models of seizures caused by picrotoxin, thiosemicarbazide, strychnine and camphor [18, 19]. Mice were randomly divided into groups of 6 animals each: 1st group – control (untreated seizures), the remaining groups – animals with modeling seizures, which were administered phenobarbital/clonazepam, as well as their combinations with digoxin.

After administration of the convulsive agent, mice were placed into individual transparent plastic cylindrical boxes and continuously monitored the condition of the animals for 1 hour (in the model of thiosemicarbazide-induced seizures – 4 hours). 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 seizures – 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 of hind limbs, 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 (240 min on the model of thiosemicarbazide-induced seizures) [18].

Classic AEDs was administered once intragastrically (i. g.) in the form of an aqueous suspension stabilized with tween-80, in conditionally effective (ED_{50}) and subeffective (1/2 ED_{50}) doses 30 min before the convulsive agent introduction: phenobarbital (Phenobarbital IC, Interchem Ukraine) – at doses of 20 and 10 mg/kg; clonazepam (Clonazepam IC, Interchem, Ukraine) – at doses of 0.1 and 0.05 mg/kg. Digoxin (DNCLZ / Health, Ukraine) was administered once subcutaneously (s. c.) at a dose of 0.8 mg/kg (1/10 LD_{50}) 10–15 min before seizure induction [14, 17, 18]. Control animals received i. g. purified water in an appropriate volume (0.1 ml per 10 g of weight).

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. have been used as convulsive agents [18, 19].

Statistical processing of the results obtained was performed using the STATISTICA 12 software package (StatSoft, USA). 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 non-parametric Mann–Whitney U-test. For the results in the alternative form (lethality, percentage of mice with clonic and tonic convulsions) the Fisher's angular transformation (φ) was used.

Results and discussion. Digoxin *per se* has a mostly moderate anticonvulsant effect under models of primary generalized seizures with different neurochemical mechanisms (Tables 1–2). Thus, cardiac glycoside probably prolongs the latency of picrotoxin-induced seizures, reducing the percentage of animals with tonic paroxysms and the duration of the seizure period, and under thiosemicarbazide-induced seizures prolongs the latency of the first convulsions compared with control. Under the strychnine-induced seizure model, digoxin not only statistically significantly prolongs the latency of the first seizures, but also significantly reduces the number of seizures in 1 mouse (due to both clonic and tonic paroxysms), reduces the severity of convulsions and the duration of seizures as well as significantly reduces animals' lethality (17 % vs. 100 % in control, $p < 0.01$). Under conditions of camphor-induced paroxysms, the anticonvulsant effect of cardiac glycoside was verified only by significant prevention of animal death (lethality – 0 %, $p < 0.01$) and reduction of clonic-tonic convulsions in 1 mouse compared with the group of animals with untreated seizures.

The results of the study of the effect of digoxin on the anticonvulsant potential of phenobarbital are presented in Table 1.

Under the conditions of picrotoxin-induced seizures, which characterized by blockade of GABA-ergic inhibition [20], phenobarbital has shown a dose-dependent anticonvulsant effect. At ED_{50} the drug provides a predictable pronounced protective effect: prevents the death of animals in the experimental group (lethality – 0 %, $p < 0.01$), statistically significant compared with control prolongs the latency period of seizures, while reducing the number of paroxysms in 1 mouse (both clonic and and tonic component), as well as reduces the severity of seizures and the duration of the convulsive period. When administered at 1/2 ED_{50}, the

Table 1

Anticonvulsant effect of digoxin, phenobarbital and their combination in the primary generalized seizures in mice

Group of animals	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				
Picrotoxin-induced seizures								
Control (untreated seizures)	13.0 ± 0.59	3.33 ± 0.56	100	100	5.0 ± 0.45	30.10 ± 5.43	49.06 ± 10.11	50
Digoxin	36.94 ± 5.79**	2.0 ± 0.52	83	67*	3.50 ± 0.81	4.88 ± 1.57**	33.40	17
Phenobarbital, ED_{50}	34.35 ± 8.47*	1.17 ± 0.60*	67*	33**	2.33 ± 0.76*	2.62 ± 2.56**	–	0**
Phenobarbital, ½ ED_{50}	27.56 ± 10.28	2.67 ± 1.09	67*	50**	3.17 ± 1.11	7.82 ± 4.36*	30.76 ± 2.39	33§
Phenobarbital, ½ ED_{50} + Digoxin	24.52 ± 1.97**, #	1.83 ± 0.17*	100§, °	17**, #	3.33 ± 0.33*	6.23 ± 2.09**	–	0**, °
Tiosemicarbaside-induced seizures								
Control (untreated seizures)	61.88 ± 4.05	2.50 ± 0.56	100	100	6.0 ± 0	17.84 ± 6.27	79.72 ± 7.27	100
Digoxin	76.33 ± 2.44*	1.50 ± 0.22	100	83	5.50 ± 0.50	8.13 ± 3.90	84.05 ± 5.12	83
Phenobarbital, ED_{50}	125.42 ± 24.37**, #	2.67 ± 0.95	83	67*	3.67 ± 0.84**	24.15 ± 11.99	109.30	17**, ##
Phenobarbital, ½ ED_{50}	64.59 ± 4.30§§	3.83 ± 0.60##	100	100§	5.83 ± 0.17§	37.30 ± 9.49#	94.73 ± 9.04	83§§
Phenobarbital, ½ ED_{50} + Digoxin	100.96 ± 8.38**, #, °	2.67 ± 0.67	100	83	5.50 ± 0.50	13.96 ± 4.26°	112.87 ± 7.45*, #	83§§
Strychnine-induced seizures								
Control (untreated seizures)	7.47 ± 0.58	1.50 ± 0.34	100	100	6.0 ± 0	1.17 ± 0.66	8.64 ± 0.81	100
Digoxin	29.67 ± 9.66**	0.67 ± 0.21*	67*	33**	2.67 ± 0.95**	0.12 ± 0.06*	11.80	17**
Phenobarbital, ED_{50}	51.75 ± 8.25**	0.33 ± 0.33*	17**, #	0**, #	0.50 ± 0.50**	0.66 ± 0.66*	–	0**
Phenobarbital, ½ ED_{50}	26.25 ± 10.71	1.17 ± 0.40	67*, §	67*, §§	3.83 ± 1.22	3.01 ± 1.44	13.42 ± 3.94	50**, §§
Phenobarbital, ½ ED_{50} + Digoxin	51.27 ± 8.73**	0.17 ± 0.17**, °	17**, #, °	0**, #, °°	0.50 ± 0.50**, °	0.03 ± 0.03*, °	–	0**, °°
Camphor-induced seizures								
Control (untreated seizures)	6.35 ± 1.55	7.33 ± 0.71	100	100	5.0 ± 0.45	49.97 ± 7.23	51.39 ± 14.06	50
Digoxin	5.74 ± 0.41	5.17 ± 0.54*	100	100	4.0 ± 0	44.19 ± 7.17	–	0**
Phenobarbital, ED_{50}	5.11 ± 1.05	2.83 ± 0.48**, #	100	100	4.0 ± 0	20.91 ± 5.41*, #	45.83	17
Phenobarbital, ½ ED_{50}	5.76 ± 1.59	3.83 ± 0.31**	100	100	4.33 ± 0.33	15.60 ± 3.32**, #	32.83	17
Phenobarbital, ½ ED_{50} + Digoxin	8.65 ± 1.65	2.83 ± 0.48**, #	100	50**,##, §§, °°	3.50 ± 0.22*	10.47 ± 3.49**, #	–	0**

*Note. n – number of animals in each group, n = 6, *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 phenobarbital at ED_{50}, §§p < 0.01 when compared with phenobarbital at ED_{50}, °p < 0.05 when compared with phenobarbital at 1/2 ED_{50}, °°p < 0.01 when compared with phenobarbital at 1/2 ED_{50}.*

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Table 2

Anticonvulsant effect of digoxin, clonazepam and their combination in the primary generalized seizures in mice

Group of animals	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				
Picrotoxin-induced seizures								
Control (untreated seizures)	13.0 ± 0.59	3.33 ± 0.56	100	100	5.0 ± 0.45	30.10 ± 5.43	49.06 ± 10.11	50
Digoxin	36.94 ± 5.79**	2.0 ± 0.52	83	67*	3.50 ± 0.81	4.88 ± 1.57**	33.40	17
Clonazepam, ED_{50}	60.0 ± 0**, ##	0 ± 0**, ##	0**, ##	0**, ##	0 ± 0**, ##	0 ± 0**, ##	–	0**
Clonazepam, ½ ED_{50}	40.85 ± 8.62**	0.83 ± 0.48*	50**, §§	33**, §	1.83 ± 0.83*	4.94 ± 4.90*	–	0**
Clonazepam, ½ ED_{50} + Digoxin	60.0 ± 0**, ##	0 ± 0**, ##	0**, ##, °°	0**, ##, °	0 ± 0**, ##	0 ± 0**, ##	–	0**
Tiosemicarbaside-induced seizures								
Control (untreated seizures)	61.88 ± 4.05	2.50 ± 0.56	100	100	6.0 ± 0	17.84 ± 6.27	79.72 ± 7.27	100
Digoxin	76.33 ± 2.44*	1.50 ± 0.22	100	83	5.50 ± 0.50	8.13 ± 3.90	84.05 ± 5.12	83
Clonazepam, ED_{50}	145.44 ± 30.70**	1.0 ± 0.37*	67*, #	67*	3.33 ± 1.12*	4.86 ± 2.95*	94.44 ± 11.16	33**, #
Clonazepam, ½ ED_{50}	87.81 ± 7.59*	1.33 ± 0.21	100§	100§	5.83 ± 0.17§	13.28 ± 8.09	96.59 ± 10.32	83§
Clonazepam, ½ ED_{50} + Digoxin	148.84 ± 30.48**, #	1.17 ± 0.48	67*, #, °	67*, °	3.33 ± 1.12*, °	21.78 ± 14.66	124.99 ± 37.76	33**, #, °
Strychnine-induced seizures								
Control (untreated seizures)	7.47 ± 0.58	1.50 ± 0.34	100	100	6.0 ± 0	1.17 ± 0.66	8.64 ± 0.81	100
Digoxin	29.67 ± 9.66**	0.67 ± 0.21*	67*	33**	2.67 ± 0.95**	0.12 ± 0.06*	11.80	17**
Clonazepam, ED_{50}	52.0 ± 8.0**	0.17 ± 0.17**	17**, #	0**, #	0.50 ± 0.50**	0.03 ± 0.03*	–	0**
Clonazepam, ½ ED_{50}	36.74 ± 10.41**	0.50 ± 0.22*	50**	50**, §§	2.67 ± 1.23*	0.29 ± 0.13	14.07 ± 0.78*	50**, §§
Clonazepam, ½ ED_{50} + Digoxin	54.39 ± 5.61**	0.17 ± 0.17**	17**, #	17**	0.83 ± 0.83**	0.11 ± 0.11*	–	0**, °°
Camphor-induced seizures								
Control (untreated seizures)	6.35 ± 1.55	7.33 ± 0.71	100	100	5.0 ± 0.45	49.97 ± 7.23	51.39 ± 14.06	50
Digoxin	5.74 ± 0.41	5.17 ± 0.54*	100	100	4.0 ± 0	44.19 ± 7.17	–	0**
Clonazepam, ED_{50}	25.91 ± 8.10*, #	2.17 ± 0.87**, #	83	67*, #	3.17 ± 0.65*	9.61 ± 5.32**, #	–	0**
Clonazepam, ½ ED_{50}	26.17 ± 10.78	1.17 ± 0.48**, ##	83	50**, ##	2.50 ± 0.81*	3.94 ± 2.47**, ##	–	0**
Clonazepam, ½ ED_{50} + Digoxin	32.36 ± 8.91**, ##	0.67 ± 0.21**, ##	67*, #	17**, ##, §	2.17 ± 0.70**, ##	0.10 ± 0.03**, ##	–	0**

*Note. n – number of animals in each group, n = 6, *$p < 0.05$ 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 clonazepam at ED_{50}, §§$p < 0.01$ when compared with clonazepam at ED_{50}, °$p < 0.05$ when compared with clonazepam at 1/2 ED_{50}, °°$p < 0.01$ when compared with clonazepam at 1/2 ED_{50}.*

anticonvulsant effect of phenobarbital is manifested only in a significant decrease in the percentage of animals with clonic and tonic paroxysms, as well as the duration of the convulsive period.

On the model of thiosemicarbazide-induced seizures, which associated with increased glutamatergic neurotransmission with parallel depletion of the cerebral pool of GABA [21], phenobarbital also has a clear dose-dependent effect. In the absolute absence of anticonvulsant action at 1/2 ED_{50}, the drug has shown a strong anticonvulsant activity at ED_{50}. This was verified both by a statistically significant lengthening of the latency of the first seizures and by a reduction in the percentage of animals with tonic convulsions as well as a reduction in the severity of paroxysms. Moreover, at ED_{50} phenobarbital significantly reduces the thiosemicarbazide-induced lethality (17 % vs. 100 % in control, $p < 0.01$).

Under conditions of glycinergic inhibition blockade induced by strychnine [22], more pronounced anticonvulsant effect is also characteristic for phenobarbital at ED_{50}. When used at a conditionally effective dose, the drug not only completely prevents lethality and the development of more severe tonic convulsions, but also significantly reduces number of seizures in 1 mouse, percentage of animals with clonic paroxysms, severity of seizures, duration of convulsive period and significantly prolongs the latency. At 1/2 ED_{50}, phenobarbital only reduces lethality, halving the number of dead animals ($p < 0.01$) and reducing the % of mice with clonic and tonic seizures by a third ($p < 0.05$) compared with control. However, the effect of a low dose of the drug is probably inferior to ED_{50}.

On the model of camphor-induced seizures with unclear mechanism [23] anticonvulsant properties of phenobarbital in both doses are limited only by a statistically significant reduction in the number of clonic-tonic paroxysms in 1 mouse, as well as a probable reduction of the convulsive period in the absence of significant influence on other indicators of experimental convulsions.

The addition of digoxin contributes to a marked increase in the anticonvulsant potential of phenobarbital at low dose (Table 1). Thus, the combination of cardiac glycoside with classical AED at 1/2 ED_{50} completely prevents animal lethality on the models of picrotoxin-, strychnine- and camphor-induced seizures. Under picrotoxin-induced paroxysms, the combination of phenobarbital + digoxin additionally has a significant positive influence on other indicators, in particular, significantly prolongs the latency of the first seizures, reduces the number of clonic-tonic attacks in 1 mouse, % of animals with tonic convulsions and period of seizures.

On the thiosemicarbazide-induced seizure model, the combined use of phenobarbital at 1/2 ED_{50} with digoxin contributes only to a statistically significant prolongation of the latency of the first attacks and lifetime to death.

The potent anticonvulsant effect, fully comparable with the action of phenobarbital at ED_{50}, is inherent in the combination of phenobarbital with digoxin under conditions of strychnine-induced seizures. In addition to complete prevention of lethality, this was verified by the absence of animals with tonic paroxysms in the group, as well as a decrease in number of seizures in 1 mouse, percentage of animals with clonic convulsions, severity of seizures, period of seizures and a marked increase in latency of the first paroxysms. According to almost all studied

indicators, the effectiveness of the combination is probably superior to monotherapy with AED at 1/2 ED_{50}.

The combined use of phenobarbital with digoxin on the camphor-induced seizure model also, in addition to complete protection of animals from death, contributes to a statistically significant reduction in the number of clonic-tonic attacks in 1 mouse, severity of paroxysms and duration of convulsive period compared with similar indicators in control group. Moreover, the combination of phenobarbital + digoxin significantly reduces the percentage of mice with severe tonic seizures – probably not only compared with the group of animals with untreated seizures, but also compared with monotherapy with cardiac glycoside and classic AED at both doses.

The results of the study of the effect of digoxin on the anticonvulsant potential of clonazepam are presented in Table 2.

Predicted pronounced anticonvulsant properties of clonazepam have been verified on the model of picrotoxin-induced seizures. Although at 1/2 ED_{50} AED shows an expressed anticonvulsant effect, which is verified by preventing mice lethality, significant reduction in number of paroxysms, their severity, duration, etc., only at ED_{50} clonazepam provides the maximum protective effect completely saving experimental animals from seizures.

Under the conditions of simulated thiosemicarbazide-induced seizures, clonazepam shows a dose-dependent anticonvulsant effect. Thus, at ED_{50} the drug not only highly reduces lethality (33 % vs. 100 % in control, $p < 0.01$), but also statistically significantly decreases the number of paroxysms in 1 mouse (both due to clonic and tonic convulsions), severity of seizures and their duration, while prolonging the latency period of seizures. At 1/2 ED_{50}, clonazepam exclusively prolongs the latency of the first paroxysms ($p < 0.05$) without a significant influence on other indicators of experimental seizures.

The anticonvulsant potential of clonazepam on the model of strychnine-induced paroxysms has been verified by the administration of the drug at both studied doses. At both doses, ED_{50} and 1/2 ED_{50}, clonazepam significantly reduces animals' lethality in experimental groups, significantly decreases the number of seizures in 1 mouse due to both clonic and tonic components, reduces the severity of convulsions, while prolonging the latency of the first seizures. Additionally, clonazepam statistically significantly compared with control reduces the duration of the convulsive period in the group (under ED_{50}), and increases the lifetime to death (under 1/2 ED_{50}). Clonazepam at a low dose is still significantly ($p < 0.01$) inferior to the effects of classical AED at conditionally effective dose in terms of the expressiveness of the effect on some analyzed seizure parameters (in particular, lethality, % of animals with tonic paroxysms).

A comparable anticonvulsant effect of clonazepam at both doses has been found on camphor-induced seizures. Thus, at both ED_{50} and 1/2 ED_{50}, clonazepam equally prevented the death of animals (lethality – 0 %, $p < 0.01$ compared with control), significantly reducing the number of paroxysms in 1 mouse, decreasing the % of animals with tonic convulsions, severity and duration of attacks. In addition, clonazepam affects the latency period of the first seizures, prolonging it statistically significantly under AED at ED_{50}, and tendentious (apparently due to high variance) in the group of animals treated with AED at 1/2 ED_{50}.

Co-administration of clonazepam at 1/2 ED_{50} with digoxin significantly enhances the anticonvulsant potential of classical low-dose AED (Table 2). On the picrotoxin-induced seizure model, a combination similar to clonazepam at ED_{50} provides the maximum protective effect, preventing the development of experimental paroxysms.

Under thiosemicarbazide-induced seizures, the efficacy of co-administration of digoxin with classical low-dose AED is not inferior to that of clonazepam monotherapy at ED_{50}, as evidenced by statistically significant prolongation of the latency period of first seizures, reduction the percentage of mice with clonic and tonic paroxysms, severity of seizures, as well as the reduction of animal lethality in the experimental group. In terms of exposure to individual indicators, the combination of clonazepam + digoxin is significantly superior to the effectiveness of monotherapy with its individual components.

Expressive anticonvulsant properties, quite comparable in the effectiveness of monotherapy with classical AED at ED_{50}, are inherent in the combination of clonazepam with digoxin on the model of seizures induced by strychnine. In addition to complete prevention of animal death, this was found to be statistically significant in terms of control by reducing the percentage of mice with clonic and tonic paroxysms in the group, as well as reducing number of seizures in 1 mouse, severity of seizures, duration of convulsive period and marked prolongation of latency. In terms of the expressiveness of the influence on animal lethality in the experimental group, the effectiveness of the combination is significantly ($p < 0.01$) superior to clonazepam monotherapy at 1/2 ED_{50}.

The addition of digoxin to clonazepam at low dose in simulated paroxysms induced by camphor, provides not only the complete protection of animals from death, but also contributes to a statistically significant prolongation of latency, as well as a reduction in number of convulsions in 1 mouse (due to both clonic and tonic), severity and duration of the convulsive period not only compared with similar indicators of the control group, but also in comparison with cardiac glycoside monotherapy. Additionally, the combination of clonazepam + digoxin significantly reduced the percentage of mice with severe tonic convulsions compared with the group of animals treated with classical AED at ED_{50}.

The difference in the expressiveness of the anticonvulsant action of classical AEDs even at conditionally effective doses on different experimental models of seizures is obviously due to the peculiarities of the mechanisms of realization of their action [24]. Thus, the pronounced anticonvulsant effect of phenobarbital and clonazepam on the model of picrotoxin-induced seizures confirms their expressive GABA-ergic properties [20]. In addition, the drugs have antiglutamatergic activity (confirmed under thiosemicarbazide-induced seizures), as well as a pronounced impact of phenobarbital and clonazepam on glycinergic neurotransmission on the model of strychnine-induced seizures [21, 22]. Also, clonazepam (to a lesser extent phenobarbital) has been shown to have modulating properties under camphor-induced paroxysms, which may be due to the influence on energy metabolism of neurons [23] and cerebral monoamine balance [25].

The moderate anticonvulsant effect of digoxin *per se*, as well as the expressive ability of the drug to potentiate the weak anticonvulsant potential of subeffective doses of phenobarbital and

clonazepam on all four experimental models of seizures may indicate multiple mechanisms of its influence on the pathogenesis of convulsions – enhancing the inhibitory effect of GABA and glycine, inhibition of the excitatory effects of glutamate, normalization of energy metabolism of neurons etc. On the other hand, given the known tropism of cardiac glycosides to the key enzyme supporting the membrane potential of excitable tissues (including neurons) – Na^+, K^+-ATPase [26–28] – it can be assumed that anticonvulsant effect of digoxin is due to the impact on the final (key) stages of excitation generalization and seizures development.

The results obtained indicate the feasibility of further in-depth study of the mechanisms of anticonvulsant potential of digoxin, in particular, establishing the role of neuronal Na^+, K^+-ATPase, endogenous digitalis-like factor, the impact on the pool of neuroactive amino acids, balance of neurotophins and cytokines etc.

Thus, experimental models of primary generalized seizures with different neurochemical mechanisms have shown that digoxin not only exhibits its own anticonvulsant properties, but also enhances the anticonvulsant potential of low doses of classical antiepileptic drugs phenobarbital and clonazepam. This shows that digoxin can be used as an adjuvant agent in complex treatment of epilepsy, as it allows to reduce the dose of widely used AEDs without decrease in the effectiveness of therapy.

Conclusion

1. The effect of the cardiac glycoside digoxin on the spectrum of anticonvulsant activity of the classic commonly used antiepileptic drugs – phenobarbital and clonazepam – has been studied.
2. Digoxin has been shown not only to have a pronounced anticonvulsant effect under conditions of primary generalized seizures induced by picrotoxin and strychnine, as well as moderate antagonism with thiosemicarbazide and camphor, but also significantly enhances the anticonvulsant potential of low doses of classical drugs with known GABAergic properties – phenobarbital and clonazepam.
3. The results obtained substantiate the expediency of further in-depth study of digoxin as an adjuvant drug in the treatment of epilepsy, in particular, its drug-resistant forms.

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Digoxin potentiates the anticonvulsant effect of low-dose phenobarbital and clonazepam under primary generalized seizures with different neurochemical mechanisms

The aim of this study – to investigate the influence of cardiac glycoside digoxin on the anticonvulsant activity of low-dose phenobarbital and clonazepam under models of primary generalized seizures with different neurochemical mechanisms.

A total of 192 random-bred male albino mice have been used. Antiepileptic drugs (AEDs) were administered 30 min before to seizure induction once intragastrically at conditionally effective (ED_{50}) and subeffective (1/2 ED_{50}) doses: phenobarbital – at doses of 20 and 10 mg/kg; clonazepam – at doses of 0.1 and 0.05 mg/kg. Digoxin was administered once subcutaneously at subcardiotonic dose of 0.8 mg/kg body weight (1/10 LD_{50}) 10–15 min before seizure induction. Picrotoxin (aqueous solution 2.5 mg/kg, subcutaneously), thiosemicarbazide (aqueous solution 25 mg/kg, intraperitoneally), strychnine (aqueous solution 1.2 mg/kg, subcutaneously), camphor (oil solution 1000 mg/kg, intraperitoneally) have been used as convulsive agents.

Digoxin has been shown not only to have a pronounced anticonvulsant effect under conditions of primary generalized seizures induced by picrotoxin and strychnine, as well as moderate antagonism with thiosemicarbazide and camphor, but also significantly enhances the anticonvulsant potential of low doses

of classical drugs with well-known GABA-ergic properties – phenobarbital and clonazepam. Synergism with phenobarbital was particularly pronounced on the strychnine- and picrotoxin-induced models of seizures, to a lesser extent in the camphor-induced model, where the combination of an AED with a cardiac glycoside provided a complete protective effect – 100 % survival. In the model of thiosemicarbazide-induced seizures, the synergism of digoxin and phenobarbital was much weaker and related only to certain seizures' indicators (statistically significant increase in the latency period of convulsions and decrease in the duration of the seizure period). Digoxin enhanced the anticonvulsant effect of a subeffective dose of clonazepam in the thiosemicarbazide- and strychnine-induced models, while cardiac glycoside did not interfere with the 100 % protective effect of clonazepam in the picrotoxin- and camphor-induced models of seizures.

The results obtained substantiate the expediency of further in-depth study of digoxin as an adjuvant drug in the treatment of epilepsy, in particular, its drug-resistant forms.

Key words: digoxin, anticonvulsant, phenobarbital, clonazepam, seizures

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Дигоксин потенціює антиконвульсивний ефект низьких доз фенобарбіталу та клоназепаму за умов первинно-генералізованих судом з різними нейрохімічними механізмами

Мета дослідження – з'ясувати вплив серцевого глікозиду дигоксину на антиконвульсивну активність низьких доз фенобарбіталу та клоназепаму на моделях первинно-генералізованих судом з різними нейрохімічними механізмами.

У дослідженні використано 192 білих безпородних мишей-самців. Протиепілептичні препарати вводили за 30 хв до моделювання судом одноразово внутрішньошлунково в умовно ефективній (ED_{50}) та субефективній (1/2 ED_{50}) дозах: фенобарбітал – відповідно 20 та 10 мг/кг; клоназепам – 0,1 та 0,05 мг/кг. Дигоксин вводили одноразово підшкірно в субкардіотонічній дозі 0,8 мг/кг (1/10 LD_{50}) за 10–15 хв до індукції судом. Як судомні агенти використовували пікротоксин (2,5 мг/кг у вигляді водного розчину підшкірно), тіосемікарбазид (25 мг/кг у вигляді водного розчину внутрішньоочеревинно), стрихнін (1,2 мг/кг у вигляді водного розчину підшкірно), камфору (1000 мг/кг у вигляді олійного розчину внутрішньоочеревинно).

Дигоксин не лише виявив виразний антиконвульсивний ефект за умов первинно-генералізованих судом, індукованих пікротоксином і стрихніном, а також помірний антагонізм з тіосемікарбазидом і камфорою, але й вірогідно поліпшував протисудомний потенціал низьких доз класичних препаратів з добре відомими ГАМК-ергічними властивостями – фенобарбіталу та клоназепаму. Щодо фенобарбіталу синергізм був особливо виразним на стрихніновій, пікротоксиновій моделях судом, меншою мірою на камфорній моделі, на яких комбінація антиконвульсанта з серцевим глікозидом забезпечила повний захисний ефект – 100 % виживаність. На моделі тіосемікарбазид-індукованих судом синергізм дигоксину та фенобарбіталу був значно слабшим і стосувався лише окремих параметрів перебігу нападів (статистично значуще збільшення латентного періоду судом і зменшення тривалості судомного періоду). Протисудомну дію субефективної дози клоназепаму дигоксин посилював на тіосемікарбазид- і стрихнін-індукованій моделях, а на пікротоксиновій і камфорній моделях судом серцевий глікозид не заважав 100 % захисному ефекту клоназепаму.

Отримані результати зумовлюють доцільність подальших поглиблених досліджень дигоксину як допоміжного засобу в лікуванні епілепсії, зокрема її фармакорезистентних форм.

Ключові слова: дигоксин, протисудомний засіб, фенобарбітал, клоназепам, судоми

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