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ANTICONVULSANT ACTIVITY OF ORIGINAL VALPROIC ACID AMINOETHERS IN CHOLINESTERASE INHIBITOR POISONING

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Introduction. Cholinesterase inhibitors present in household chemicals, agrochemicals, and a number of medicinal products represent the most common cause of acute intoxications accompanied by the development of convulsive syndrome. Delayed and repeated administration of existing antidotes proves ineffective. Compounds that are promising for the development of alternative therapeutic agents include derivatives of valproic acid.

Objective. Evaluation of the anticonvulsant efficacy of original valproic acid aminoethers in intoxication with phenylcarbamate as a cholinesterase inhibitor.

Materials and methods. Experiments were conducted using outbred white male rats aged 3 months with a body weight of 200–240 g. The tabular express method by Prozorovsky was used to determine the median lethal doses of the new compounds. To model the convulsive syndrome, phenylcarbamate was administered intraperitoneally to male rats at a dose of 1 mg/kg bw. The anticonvulsant activity of valproic acid aminoethers — N-methyl-4-piperidinol (VAA), quinuclidinol (QVA), and tropinol (TVA) — was assessed. The preparations were administered at doses of 21.5 mg/kg bw and 43 mg/kg bw after the onset of convulsions. The study was conducted using four experimental groups: phenylcarbamate — P (n = 8), P+VAA (n = 16), P+TVA (n = 16), and P+QVA (n = 16). The test substances were dissolved in 0.9% sodium chloride solution and administered intraperitoneally, taking interspecies dose conversion into account. The volume of the intraperitoneally administered solution was 0.1 mL/100 g. The severity of the convulsive syndrome in the experiment was assessed using the Racine scale. The following efficacy indicators were taken into account: latent period, severity and duration of convulsive syndrome, and mortality. Statistical processing of the research results was performed using the Statistica 13.0 software package (Statsoft, USA).

Results. The established LD_{50} values of the original valproic acid aminoethers under study correspond to class 3 of moderately toxic substances. At a dose of 21.5 mg/kg bw, the proportion of rats with severe convulsions significantly decreased in all groups; the fastest anticonvulsant effect was recorded in the QVA group (after 10 min, convulsions were absent). The efficacy of VAA and TVA at a dose of 43 mg/kg bw was comparable to the dose of 21.5 mg/kg bw; in the QVA group, the proportion of animals with convulsions remained high after 10 min. A significant reduction in the duration of convulsions was revealed in the QVA group at doses of 21.5 mg/kg bw and 43 mg/kg bw. A significant decrease in the intensity of convulsions was detected in the VAA and QVA groups at a dose of 21.5 mg/kg bw, and at a dose of 43 mg/kg bw in the VAA and TVA groups.

Conclusions. The new aminoethers of valproic acid exhibit anticonvulsant activity in intoxication with a reversible cholinesterase inhibitor. At a dose of 21.5 mg/kg bw, QVA is the most effective; however, at a dose of 43 mg/kg bw, manifestations of toxicity are observed and VAA is more effective. Despite animal mortality, TVA also demonstrates its efficacy at a dose of 43 mg/kg bw.

Keywords: valproic acid aminoethers; convulsive syndrome; cholinesterase inhibitors; carbamates; anticonvulsant therapy

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ПРОТИВОСУДОРОЖНАЯ АКТИВНОСТЬ ОРИГИНАЛЬНЫХ АМИНОЭФИРОВ ВАЛЬПРОЕВОЙ КИСЛОТЫ ПРИ ИНТОКСИКАЦИИ ИНГИБИТОРОМ ХОЛИНЭСТЕРАЗ

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Введение. Наиболее распространенной причиной острых интоксикаций, сопровождающихся развитием судорожного синдрома, являются ингибиторы холинэстераз в составе бытовой химии, агрохимикатов и ряда лекарственных средств. Отсроченное

и повторное применение существующих антидотов малоэффективно. К соединениям, перспективным для разработки альтернативных средств терапии, относятся производные вальпроевой кислоты.

Цель. Оценка противосудорожной эффективности оригинальных аминоэфиров вальпроевой кислоты при интоксикации ингибитором холинэстеразы — фенилкарбаматом.

Материалы и методы. Эксперименты проведены на беспородных белых крысах-самцах возрастом 3 месяца и массой тела 200–240 г. При определении средних летальных доз новых соединений использовали табличный экспресс-метод по В.Б. Прозоровскому. Для моделирования судорожного синдрома внутрибрюшинно вводили крысам-самцам фенилкарбамат в дозе 1 мг/кг м.т. Оценивали противосудорожную активность аминоэфиров вальпроевой кислоты: N-метил-4-пиперидинольный (АВК), хинуклидинольный (ХАВК) и тропиновый (ТАВК), вводимые в дозах 21,5 и 43 мг/кг м.т. после начала судорог. Исследование проведено на 4 опытных группах: фенилкарбамат «Ф» (n = 8), Ф+АВК (n = 16), Ф+ТАВК (n = 16), Ф+ХАВК (n = 16). Исследуемые субстанции растворяли в 0,9%-ном растворе хлорида натрия и вводили внутрибрюшинно, с учетом межвидового пересчета доз. Объем вводимого внутрибрюшинно раствора составлял 0,1 мл/100 г. Выраженность судорожного синдрома в эксперименте оценивали по шкале Racine. Учитывали показатели эффективности: латентный период, выраженность и продолжительность судорожного синдрома, летальность. Статистическую обработку результатов исследования производили с помощью пакета программы Statistica 13.0 (Statsoft, США).

Результаты. Установленные значения $ЛД_{50}$ оригинальных аминоэфиров вальпроевой кислоты соответствуют 3-му классу умеренно токсичных веществ. В дозе 21,5 мг/кг м.т. значимо уменьшалась доля крыс с выраженными судорогами во всех группах, наиболее быстрый противосудорожный эффект регистрировали в группе ХАВК (через 10 мин судороги отсутствовали). Эффективность АВК и ТАВК при использовании в дозе 43 мг/кг м.т. была сопоставима с дозой 21,5 мг/кг м.т., в группе ХАВК через 10 мин доля животных с судорогами оставалась высокой. Достоверное уменьшение продолжительности судорог выявлено в группе ХАВК в дозах 21,5 и 43 мг/кг м.т. Достоверное снижение интенсивности судорог выявлено в группах АВК и ХАВК в дозе 21,5 мг/кг м.т., группах АВК и ТАВК — в дозе 43 мг/кг м.т.

Выводы. Новые аминоэфиры вальпроевой кислоты проявляют противосудорожную активность при интоксикации обратимым ингибитором холинэстераз. В дозе 21,5 мг/кг м.т. наиболее эффективен ХАВК, однако в дозе 43 мг/кг м.т. наблюдаются проявления токсичности и более эффективен АВК. Несмотря на летальность животных, ТАВК также проявляет свою эффективность в дозе 43 мг/кг м.т.

Ключевые слова: аминоэфиры вальпроевой кислоты; судорожный синдром; ингибиторы холинэстераз; карбаматы; противосудорожная терапия

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Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Проведение исследований одобрено на заседании биоэтического комитета ФГБУ НКЦТ им. С.Н. Голикова ФМБА России (протокол № 1/22 от 22.02.2022).

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INTRODUCTION

Poisoning with cholinesterase inhibitors remains the primary cause of generalized convulsive syndrome of toxic etiology. Substances in this group include irreversible inhibitors, primarily organophosphorus compounds (OPCs), and reversible inhibitors, such as derivatives of carbamic acid (carbamates). Carbamates and OPCs are used as components of agricultural and household chemicals (pesticides, insecticides), as well as plasticizers and polymeric materials. Carbamates are also widely applied in the pharmaceutical industry as part of medicinal products [1, 2]. The number of victims of poisoning with cholinesterase inhibitors (household poisonings, agrochemical poisonings, drug overdoses, or suicides) amounts to several million per annum worldwide.

The toxic effects of cholinesterase inhibitors on the body are manifested in the development of miosis, bronchospasm, hypersecretion, vomiting, arrhythmia, and respiratory failure. These conditions result from enhanced muscarinic and nicotinic stimulation due to the inhibition of acetylcholinesterase (AChE) activity. This inhibition promotes the accumulation of acetylcholine in neuronal synapses and the development of cholinergic syndrome [3]. Additionally, butyrylcholinesterase, carboxylesterase, and some other enzymes are inhibited [1, 4, 5]. At the level of the central nervous system (CNS), intoxication effects include generalized convulsive seizures, which can last for more than 30 min and involve significant damage to neurons and neuroglial cells in the brain. Furthermore, cholinergic manifestations are supplemented by glutamatergic excitotoxicity, progression of neuroinflammation and neurodegeneration. Victims

may experience persistent neurological disorders for an extended period of time [6].

It appears evident that a timely and effective suppression of convulsions is essential for improving the survival rates after acute poisoning with cholinesterase inhibitors, as well as for ensuring neuroprotection and prevention of CNS dysfunction in the long-term post-intoxication period. However, the existing antidote therapy is primarily aimed at reducing severity and preventing fatalities in the earliest stages of intoxication (prior to the onset of convulsions).

The effectiveness of antidote therapy decreases significantly when administered during actively developing or established convulsive syndrome. In particular, atropin [5] acts only on muscarinic, rather than on nicotinic, cholinergic receptors [6]. This compound is most effective when administered prophylactically or within the first minutes after exposure. Oximes, such as pralidoxime, etc., are recommended as prophylactic antidotes and exclusively for organophosphorus compound (OPC) poisoning [4]. Among benzodiazepines, which interact with gamma-aminobutyric acid (GABA) receptors, midazolam is preferred due to its rapid action, attributed to its high penetrative properties across the blood–brain barrier (BBB) [5]. However, their efficacy may be significantly reduced due to a substantial decline in the expression of receptor subunits and structurally associated proteins and enzymes capable of interacting with these substances within 10–20 min after exposure [6]. This greatly increases the share of refractory (antidote-resistant) convulsions.

The above-mentioned considerations underscore the necessity for developing new effective means to suppress convulsive syndrome in poisoning with cholinesterase inhibitors. One promising direction consists in the development of anticonvulsant agents based on derivatives of valproic acid. It is known that valproic acid participates in pre- and postsynaptic modulation of GABAergic signaling, affects sodium, calcium, and potassium channels, can increase extracellular levels of serotonin and dopamine in the hippocampus, modulate neurogenesis, and exert a neuroprotective effect [7]. However, the high effective dose of this anticonvulsant for generalized convulsive syndrome (over 150 mg/kg bw) limits its use as an antidote. Moreover, high doses of valproic acid are associated with a teratogenic effect [8].

With the purpose of reducing the effective dose, new pharmaceutical agents based on valproic acid are being developed. For example, valpromide (VPM), a recently developed substance, was shown to act as a prodrug (valproic acid is released during hydrolysis in the stomach) [8, 9]. However, VPM, approved only in France and Italy (Depamide®), is recommended for use exclusively in bipolar disorders (not as an anticonvulsant) [9]. Valnoctamide (VCD) — an isomer of valpromide — with its own therapeutic activity (biotransformation with the release of valproic acid in the body is minimal) has been developed. Currently, VCD has successfully passed phase IIb of clinical trials and is included in the list of sedative agents as an anticonvulsant [8]. Sec-butylpropylacetamide (SPD), a recently synthesized homologue of VCD, showed high anticonvulsant activity in various experimental models in preclinical studies, including cases of benzodiazepine-resistant convulsions [8]. The efficacy of SPD is due to a significantly faster rate of penetration through the blood–brain barrier (12 times faster than valproic acid). SPD was demonstrated to preserve cognitive functions and reduce neuronal damage [10]. However, SPD and VCD are poorly soluble in water and are used in the form of an emulsion [11], which is inconvenient for use as an antidote. All the above-mentioned derivatives of valproic acid are not registered as agents for the relief of generalized convulsive syndrome.

The aim of the study is a comparative investigation of the anticonvulsant efficacy of original valproic acid aminoether substances in poisoning with a cholinesterase inhibitor — phenylcarbamate.

MATERIALS AND METHODS

Original valproic acid derivatives were developed and synthesized at the Laboratory of Drug Synthesis of the Golikov Federal Research Center of Toxicology. The list and structural formulas of the tested substances are presented in Table 1.

In the experiments, outbred white male rats aged three months and weighing 200–240 g were used as the test system. The animals were obtained from the nursery of the National Research Center “Kurchatov

Table 1. Molecular characteristics of valproic acid aminoethers

Name	Gross formula	Purity (%)
(1-Methylpiperidin-4-yl) 2-propylpentanoate hydrochloride (VAA)	$C_{14}H_{27}NO_2$*HCl	98.27
1-Azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate (QVA)	$C_{15}H_{27}NO_2$*HCl	98.03
8-Methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate (TVA)	$C_{16}H_{29}NO_2$*HCl	98.09

Table compiled by the authors

Institute" — RAPPOLOVO Laboratory Animal Breeding Facility (Leningrad Oblast). The animals were kept under standard conditions in accordance with the rules[1].

At the first stage, an assessment of acute toxicity and determination of the median lethal dose (LD_{50}) for TVA and QVA was conducted using the express method by Prozorovsky [12]. In order to study each compound, the experimental animals were divided into four groups of two rats each; a single intraperitoneal injection of the test solutions was performed (the substances were dissolved in 0.9% sodium chloride solution). The following doses were selected for the acute toxicity study of TVA and QVA substances: QVA — 63.1, 79.4, 100.0, 126.0 mg/kg; TVA — 79.4, 100.0, 126.0, 158.0 mg/kg. Previous studies had determined the LD_{50} for VAA to be 170 ± 1.2 mg/kg bw [13].

At the second stage, the pharmacological activity of the substances was studied using a model of convulsions induced by a reversible cholinesterase inhibitor — substituted 2[(dimethylamino)methyl] aryldimethylcarbamate hydrochloride (hereinafter referred to as phenylcarbamate) [14]. Phenylcarbamate was administered as a single intraperitoneal injection at a dose of 1 mg/kg bw [15]. The following experimental groups were formed: a group with isolated administration of phenylcarbamate "P" as the convulsive agent (n = 8), and three groups with administration of "P" followed by administration of the test corrective agents: P+VAA (n = 16), P+TVA (n = 16), P+QVA (n = 16). The anticonvulsant efficacy of the VAA, QVA, and TVA substances was assessed at two doses — 21.5 mg/kg bw and 43.0 mg/kg bw (eight animals per dose for each test substance).

The selection of effective doses of the test substances and the administration regimen was based on the dosage and usage protocols of sodium valproate (Convulex®) adopted in clinical practice for humans. The average dose of 7 mg/kg bw was used as the basis for calculating the effective dose. Interspecies dose conversion from human to rat was performed using the standard recommendations of Mironov[2]. The test dose values were calculated using the following coefficients: the therapeutic dose for rats was 7.0 × 39 (coefficient for a human weighing 70 kg) / 6.5 (coefficient for a rat weighing 200 g) ≈ 43.0 mg/kg bw.

The test substances were dissolved in a 0.9% sodium chloride solution and administered intraperitoneally, taking interspecies dose conversion into account. The volume of the intraperitoneally administered solution was 0.1 mL/100 g. Administration was performed within the first minutes after the onset of seizures at levels 3–4 on the Racine scale [16], which were induced by the administration of the cholinesterase inhibitor.

The following efficacy indicators were taken into account: latent period, severity and duration of convulsive syndrome, and mortality. Observation and recording of lethal outcomes were conducted over a 24-h period. Mortality was assessed based on the proportion of deceased rats relative to their total number in the study group after the administration of the convulsive agent during the 24-h observation period. The severity of convulsive syndrome in the experiment was assessed using the Racine scale. Seizures of level 4 and above, equivalent to generalized clonic-tonic seizures in humans, were classified as severe. The duration of convulsive syndrome was measured in minutes.

To assess the significance of differences in the frequency of rats exhibiting severe seizures, Fisher's exact test was used. A comparative assessment of the convulsive syndrome indicators in male rats poisoned with phenylcarbamate was performed using the Kruskal–Wallis test. To identify differences between individual groups, as well as between the studied substances and the "P" group, Dunn's multiple comparison test (post-hoc analysis) was employed. Statistical processing of the research results was carried out using the Statistica 13.0 software package (Statsoft, USA).

RESULTS

In the course of work to determine the quantitative characteristics of acute toxicity upon intraperitoneal administration of QVA, the following distribution of rat mortality in each dose subgroup was established: 0, 0, 2, 2 individuals, which allowed for the determination of the LD_{50} for QVA at a level of 89.8 ± 7.1 mg/kg; upon intraperitoneal administration of TVA — 0, 0, 2, 2 individuals, which corresponded to an LD_{50} of 113.1 ± 8.9 mg/kg. Based on the obtained LD_{50} values of the original valproic acid aminoethers, the compounds can be classified as class 3 moderately toxic substances.

The experimental model of seizures induced by a reversible acetylcholinesterase inhibitor (phenylcarbamate) [12, 13] following the administration of VAA, QVA, and TVA at two doses (21.5 mg/kg bw and 43.0 mg/kg bw) revealed no statistically significant difference between the share of deceased rats in the group of animals receiving the convulsive agent, regardless of the dose and the recorded time interval. During the experiment, the death of one animal in the "P" group at the 30-min observation mark, the death of one rat following the administration of TVA at a dose of 21.5 mg/kg bw at the 10-min observation mark, and the death of three rats in the QVA group at a dose of 43.0 mg/kg bw at the 20-min observation mark were recorded.

After the administration of phenylcarbamate, the number of rats with severe convulsions in the "P" group began to decrease from the 30-min observation point by 14%; by 70 min, convulsions were no longer recorded in the animals.

[1] SP 2.2.1.3218-14 dated September 28, 2014 "Sanitary and Epidemiological Requirements for the Design, Equipment, and Maintenance of Experimental-Biological Clinics (Vivaria)".

[2] Mironov AN. Guidelines for conducting preclinical drug trials. Part 1. Moscow: Grif&K; 2012.

Table 2. Effect of the investigated drugs on the occurrence of seizures in rats following administration of phenylcarbamate and valproic acid aminoether substances

Group	Time intervals, min								
	0	5	10	20	30	40	50	60	70
Number of rats (*n/N*) after administration of substances at a dose of 21.5 mg/kg b.w.									
P	8/8	8/8	8/8	8/8	6/7	5/7	3/7	1/7	0/7
P+VAA	8/8	8/8	3/8*	0/8*	0/8*	0/8*	0/8	0/8	0/8
P+TVA	8/8	8/8	0/7*	3/7*	1/7*	0/7*	0/7	0/7	0/7
P+QVA	8/8	8/8	0/8*	0/8*	0/8*	0/8*	0/8	0/8	0/8
Number of rats (*n/N*) after administration of substances at a dose of 43.0 mg/kg b.w.									
P	8/8	8/8	8/8	8/8	6/7	5/7	3/7	1/7	0/7
P+VAA	8/8	8/8	2/8*	0/8*	0/8*	0/8*	0/8	0/8	0/8
P+TVA	8/8	8/8	1/8*	1/8*	0/8*	0/8*	0/7	0/7	0/7
P+QVA	8/8	8/8	8/8	0/8*	0/8*	0/8*	0/8	0/8	0/8

Table compiled by the authors based on their own data

Note: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate; *n* — number of rats with severe convulsions; *N* — total number of rats in the group; * — differences are statistically significant compared to the P group ($p \leq 0.05$).

The administration of the original aminoethers reduced the number of animals with convulsion severity of level 4 and above (Racine scale), which corresponds to a generalized convulsive syndrome in humans (Table 2).

Following the administration of the test substances at a dose of 21.5 mg/kg bw, a statistically significant reduction in the proportion of rats with severe convulsions was observed during the 10–40 min period of observation (Table 2).

In the P+QVA group, animals with severe convulsions were completely absent starting from the 10-min observation mark, whereas in the other two groups, the anticonvulsant effect occurred later. Specifically, in the P+TVA group, no severe convulsions were observed at 10 min; however, cases of clonic-tonic convulsions were recorded over the next 20 min, which ceased by 40 min. In the P+VAA group, a reduction in convulsion severity occurred by the 20-min observation mark.

No statistically significant intergroup differences were identified when comparing animals that received different substances at the same time interval.

When the substances were administered at a dose of 43.0 mg/kg bw, the anticonvulsant efficacy in the P+VAA and P+TVA groups generally coincided with that established at the lower dose. However, in the P+QVA group, 10 min after substance administration, the number of animals with severe convulsions did not decrease compared to the “P” group. At the same time, by 10 min, in the P+VAA and P+TVA groups, the number of animals with convulsive syndrome was statistically significantly lower than that in the group with isolated administration of phenylcarbamate.

When assessing the total duration of convulsions using statistical processing of the results with the Kruskal–Wallis test, the presence of statistically significant differences between all studied groups upon administration of the substances at both doses was revealed (Table 3).

When constructing a quadratic matrix of post-hoc comparisons (*p*-values) for the duration of convulsions following intraperitoneal administration of the test substances and in the phenylcarbamate group using Dunn’s post-hoc test, no statistically significant intergroup differences were identified. However, when comparing the duration of convulsions in the P+QVA group (dose 21.5 mg/kg bw) with the values in the “P” group, a statistically significant reduction in the median (Me) duration of convulsions by 77% was detected ($p = 0.001$). A lower median duration of convulsions by 57% was also recorded when comparing the indicator in the P+QVA and P+VAA groups at the dose level of 21.5 mg/kg bw ($p = 0.041$).

When the substances were administered at a dose of 43.0 mg/kg bw, differences in the duration of convulsions were established, comparable to the results obtained when studying the effects of lower doses. Specifically, significant differences were found when

Table 3. Comparative assessment of convulsion duration in rats after intraperitoneal administration of phenylcarbamate and valproic acid aminoether substances at doses of 21.5 and 43.0 mg/kg bw

Group	Dose of valproic acid aminoethers	Animals amount	Convulsion duration, min			*p*-value of intergroup differences[1]
		N	M_e	Min	Max	
P	–	8	87.0	13.0	103.0	0.003
P+VAA	21.5 mg/kg b.w.	8	46.5	34.0	57.0	
P+TVA		8	40.0	8.0	44.0	
P+QVA		8	20.0	20.0	20.0	
P	–	8	87.0	13.0	103.0	0.002
P+VAA	43.0 mg/kg b.w.	8	70.0	31.0	81.0	
P+TVA		8	48.0	45.0	50.0	
P+QVA		8	30.0	12.0	32.0	

Table compiled by the authors based on their own data

Note: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate; «–» — isolated administration of phenylcarbamate; [1] — *p*-value in the Kruskal-Wallis test.

Table 4. Comparative assessment of the time-weighted sum of seizure intensity scores in male rats after intraperitoneal administration of valproic acid aminoether substances at doses of 21.5 and 43.0 mg/kg bw

Group	Dose of valproic acid aminoethers	Number of animals	Time-weighted sums of seizure intensity scores, score/min			p-value of intergroup differences[1]
			M_e	min	max	
P	–	7	270	210	310	0.001
P+VAA	21.5 mg/kg bw	8	61	53	98	
P+TVA		7	85	48	115	
P+QVA		8	19	18	20	
P	–	7	270	210	310	0.001
P+VAA	43.0 mg/kg bw	8	110	63	183	
P+TVA		8	66	65	165	
P+QVA		5	78	78	90	

Table compiled by the authors based on their own data

Note: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate; «–» — isolated administration of phenylcarbamate; [1] – *p*-value in the Kruskal–Wallis test.

comparing the P+QVA group with the phenylcarbamate group ($p = 0.001$) and in the intergroup comparison between P+QVA and P+VAA ($p = 0.039$).

Time-weighted sums of seizure intensity scores (area under the curve "seizure score–time," AUC) were calculated. Deceased individuals were excluded from the calculations. It was demonstrated (Table 4) that there are statistically significant differences between the AUC indicators upon administration of the substances at doses of 21.5 mg/kg bw ($p = 0.001$) and 43.0 mg/kg bw ($p = 0.001$).

Upon administration of the substances at a dose of 21.5 mg/kg bw, the time-weighted sums of seizure intensity scores in the P+VAA group were found to be statistically significantly lower by 77% than the corresponding indicator in the phenylcarbamate group ($p = 0.041$), and in the P+QVA group — by 93% ($p = 0.001$) based on the median, respectively. In the P+TVA group, the

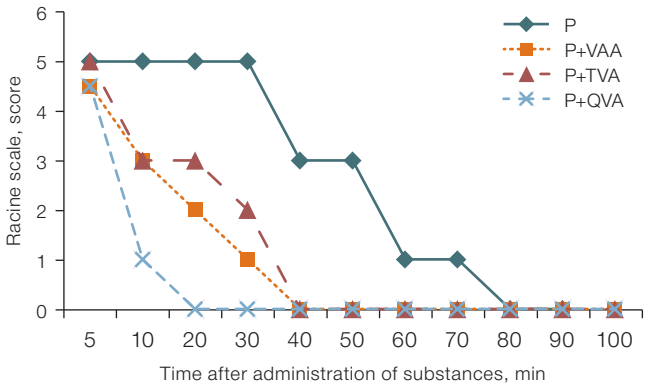

Figure prepared by the authors based on their own data

Fig. 1. Median-based plots of the severity of the convulsive syndrome according to the Racine scale (points) versus time after administration of valproic acid aminoether substances at a dose of 21.5 mg/kg bw to male rats: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate

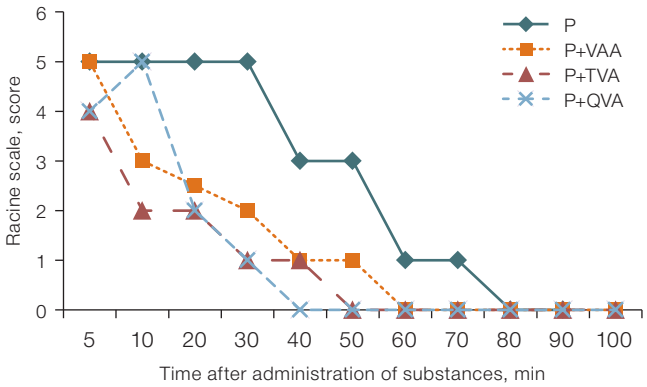

Figure prepared by the authors based on their own data

Fig. 2. Median-based plots of the severity of the convulsive syndrome according to the Racine scale (points) versus time after administration of valproic acid aminoether substances at a dose of 43 mg/kg bw to male rats: P — Phenylcarbamate; VAA — (1-methylpiperidin-4-yl) 2-propylpentanoate hydrochloride; QVA — 1-azabicyclo[2.2.2]oct-3-yl 2-propylpentanoate; TVA — 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-propylpentanoate

AUC level was lower by 84%, although not reaching statistical significance compared to the "P" group. A statistically significant decrease in the AUC indicator was also recorded in the P+QVA group compared to the P+TVA group, with a difference of 78% (p = 0.026).

When the substances were administered at a dose of 43.0 mg/kg bw, a significant reduction in the median AUC indicator was noted: by 59% (p = 0.015) in the P+VAA group and by 79% in the P+TVA group (p = 0.001) compared to the animals that received only phenylcarbamate.

Figures 1 and 2 show the dynamics of the median scores for the severity of convulsive syndrome (from 0 to 6 points on the Racine scale) after administration of the test substances over a 100-min observation period. Administration of the QVA substance at a dose of 21.5 mg/kg bw demonstrated the most favorable time course for the severity of convulsive syndrome, corresponding to the greater anticonvulsant efficacy of this compound. Conversely, when a dose of 43.0 mg/kg bw was administered, more preferable changes were observed in animals that received VAA and TVA.

DISCUSSION

Our results indicate that the administration of original valproic acid aminoethers in the setting of acute intoxication with a cholinesterase inhibitor contributed to a pronounced reduction in the duration of convulsions within the first 10–20 min after the onset of exposure. The highest efficacy was demonstrated by QVA at a dose of 21.5 mg/kg bw. This dose led to cessation of convulsions in 100% of animals after 10 min.

The time-weighted sum of seizure intensity scores (AUC) in the groups receiving the original valproic acid aminoethers at both doses was significantly lower compared to the group of animals without therapy (only phenylcarbamate administration), indicating sufficient efficacy of the test substances in reducing the severity of not only intense but also other types of convulsions. Overall, the lowest median AUC value (corresponding to the highest efficacy) was established for QVA (valproic acid quinuclidinol aminoether) when administered at a dose of 21.5 mg/kg bw. The VAA and TVA samples at the studied doses were comparable to each other in terms of efficacy.

Although no statistically significant differences in mortality were detected between the studied groups across all time intervals, the identified death cases of three animals at the 20-min mark of the experiment following the use of QVA at a high dose (43.0 mg/kg bw) require further investigation. It is evident that these cases could have been associated with either the toxicity of QVA or the individual reaction of specific animals to phenylcarbamate exposure, independent of QVA administration. Furthermore, the pronounced efficacy of QVA at a dose of 21.5 mg/kg bw suggests that a further dose reduction without efficacy loss is possible and that the 43 mg/kg bw dose may be excessive. It has been previously established that the anticonvulsant efficacy of valpromide in experiments on mice is 3–5 times higher than that of valproic acid [9]; it is possible that in the case of QVA, the optimal dose will also be significantly lower and not associated with any manifestations of toxicity.

Previous animal models demonstrated that, in comparision with atropine — a standard antidote for intoxication with cholinesterase inhibitors [17], VAA is more effective in reducing the duration of convulsive syndrome. In this light, the developed original valproic acid derivatives should be considered promising for further study and the development of agents to terminate the convulsive syndrome induced by cholinesterase inhibitors, including irreversible ones.

CONCLUSIONS

1. The developed original valproic acid derivatives (VAA, QVA, and TVA) are effective in relieving convulsive syndrome developing due to poisoning with cholinesterase inhibitors and can be used for further study as potential medicinal products.

2. In terms of all the parameters studied, the QVA substance exhibited the highest anticonvulsant efficacy at a dose of 21.5 mg/kg bw. However, when using a dose of 43 mg/kg bw, the VAA substance demonstrated a more effective relief of the convulsive syndrome. The TVA substance exhibited its anticonvulsant efficacy when used at the increased dose of 43 mg/kg bw.

3. Further selection of effective doses and investigation of the toxicity of the valproic acid quinuclidinol aminoether (QVA) are necessary.

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