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Neuroprotective activity of 2-methyl-3-phenylaminomethylquinolin-4-one in experimental traumatic brain injury in rats

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ABSTRACT

Current research has been undertaken to investigate neuroprotective effects of novel antidepressant atristamine (2-methyl-3-phenylaminomethylquinolin-4-one) on brain damage after traumatic brain injury (TBI) in rats. It has been shown that treatment with atristamine (100 mg/kg) has protective effects against TBI. This has been demonstrated by reduced neurological deficit using McGraw scale, improved indicators of orientational-exploratory activities and emotional reactions in open field test, enhanced muscle tone and coordination of movements in vertical screen test, reduced level of anxiety in elevated plus maze. Also researched substance had positive impact on cognitive functions in extrapolation escape task and did not impair physical endurance in weight-loaded forced swimming test. Atristamine showed significant antidepressant activity in forced swimming test, confirming his competence as an antidepressant in pathological state. It has been disclosed that atristamine did not improve the conditions of experimental animals in the model of bilateral common carotid artery occlusion. Thus, the results prove that atristamine exhibits neuroprotective activity against TBI that, in conjunction with high antidepressant activity, could extend application in clinical practice.

Keywords: 2-methyl-3-phenylaminomethylquinolin-4-one, neuroprotective activity, traumatic brain injury, antidepressant, behavioral tests.

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of disability in all regions of the world. It constitutes 30-40% of all injuries and takes the first place among indicators of mortality and invalidization among people of working age. According to WHO (World Health Organization), over 10 million people affected annually by TBI [1]. Furthermore, the burden of TBI is manifest throughout the world in the course of time that actualizes a search of novel and more effective drugs to reduce unnecessary suffering due to brain trauma.

TBI launches a number of different pathobiochemical humoral responses that indirectly enhance injury of cerebral tissue caused by direct mechanical influence [2]. Since there are no means for correcting the primary chain of pathogenesis – biomechanical alterations of brain tissue, the main direction of pharmacotherapy is prevention of the development of secondary processes including release of neurotransmitters, generation of free radicals, calcium-mediated injuries, activation of genes, dysfunction of mitochondria and inflammatory responses [3]. For the purpose of protection of brain cells from death different groups of drugs are widely used [4].

One of the most common and socially important long-term consequences of TBI is depression [5]. Triggers of depressive disorders may include biological, psychological and social factors, but special attention should be paid to biological factors due to direct association with damage of the brain [6], and as a result, malfunction of neurotransmitter systems. The dependence between occurrence of depression after TBI and anatomical localization

of injury that has been proved in many studies speaks in favour of this fact [7]. Consequently, in the long-term perspective patients with depressive disorders caused by TBI widely use antidepressants [6]. Most drugs of this group have neuroprotective properties that are associated with activation of MAPK/ERK and Wnt/GSK signal cascades [8], but their protective mechanisms have background and deferred character. This is why, the development of new safe drugs that can be used for treatment from the first hours after TBI both to neuroprotection and prevention of possible depressive disorders remains relevant.

High antidepressant activity of atristamine (2-methyl-3-phenylaminomethylquinolin-4-one, figure 1) has been proved in previous researches [9, 10].

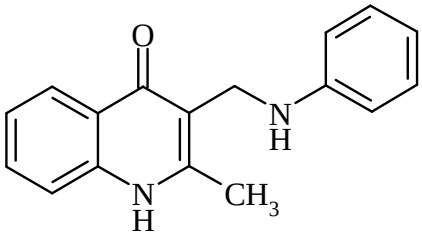

Figure 1. Chemical structure of atristamine (2-methyl-3-phenylaminomethylquinolin-4-one)

Furthermore, a unique spectrum of additional neuropharmacological properties of this molecule (antihypoxic, anti-amnesic, alcoprotective) has been investigated [11-13]. Using ELISA method, it has been shown that after treatment with atristamine significant diminution of serotonine concentration harmonizes with increased level of dopamine and epinephrine in mice's brain [14]. Thus, the results of the own experiments were the basis for the study of the neuroprotective properties of atristamine that, in conjunction with high antidepressant activity, could extend application in clinical practice.

Thus, the purpose of current research was to investigate the neuroprotective properties of atristamine in the model of mild TBI in rats using applicable behavioral tests.

EXPERIMENTAL SECTION

Chemicals

2-Methyl-3-phenylaminomethylquinolin-4-one has been synthesized from 2-methyl-1H-quinolin-4-one via aminomethylation and further interaction of the Mannich base obtained with aniline [15]. Piracetam (200 mg/ml, injection) has been used as standard drug in this study.

Experimental animals

The experiment has been performed on 40 female albino rats weighting 200-240 g in compliance with bioethical requirements in accordance with the "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" [16]. All experimental animals were kept on the appropriate food diet according to the standard health and safety regulations in the vivarium at the Central Research Laboratory of the National University of Pharmacy that certified by the State Enterprise "Centre for Drug Evaluation and Research at the Ministry of Public Health of Ukraine" as a base for research in experimental pharmacology.

Induction of mild TBI

Mild TBI was induced by weight-drop technique applying a dosed impact to the parietal-occipital area of skull by free fall of weight (0.05 kg) from the height of 0.5 m under light ether anesthesia [17].

Experimental protocol

The behavioral tests that would allow thoroughly evaluate the protective effects on the functions of the CNS after mild TBI were used for revealing neuroprotective properties of atristamine. The protocol of the experiment included two stages. Firstly, all groups of animals were examined in corresponding tests before TBI in order to identify the initial parameters. Then, in the same order all techniques were repeated after induction of the pathology. Considering duration of the tests, each stage was supposed to last for 2 days: the first day – open field test (OFT) [18], elevated plus maze (EPM) [19], vertical screen test (VST) [20]; the second day – extrapolation escape task (EET) [21], forced swimming test (FST) (or Porsolt "behavioral despair" test) [22] and weight-loaded forced swimming test (WFST) [23]. The animals' neurological deficit during next days after TBI was assessed using McGraw scale modified by I.V. Gannushkina [24].

All animals were divided randomly into four experimental groups depending on the treatment protocol:
1) intact group (n=6) – rats were anesthetized without TBI induction;
2) disease group (n=7) – rats were anesthetized with TBI induction;
3) atristamine group (n=7) – rats after TBI treated with atristamine in dose 100 mg/kg;
4) piracetam group (n=6) – rats after TBI treated with piracetam in dose 400 mg/kg.

Animals received appropriate agents that were applied intragastrically (atristamine as stable suspension and piracetam as water solution) 30 minutes before induction of TBI and during next two days 30 minutes before the tests. The intact and the disease groups were administered with saline in the same order.

Statistical analysis

Mean values (M) ± standard deviation (SD) have been used for statistical significance calculations. Statistical analysis of the results has been carried out by methods of variation statistics. Intergroup differences have been calculated by the independent Student t-test. Differences between initial and posterior parameters have been assessed using the dependent Student t-test for paired samples. Also nonparametric methods of analysis (Fischer exact test) have been used. A p-value of ≤0.05 has been considered significant.

RESULTS AND DISCUSSION

Open field test

The results of the primary research of animals' behavior in the OFT revealed initial parameters for each group. Also the absence of significant intergroup differences of the animals in the intact state was proved (Table 1).

On the second day after the induction of mild TBI in the intact group the trend towards decrease of all parameters was observed. This fact can be explained by the influence of ether anesthesia and the effect of "habituation". In the disease group after injury the same trend took place, but it had more expressive character: the number of explored holes significantly decreased by 2.9 times ($p<0.05$) that correlates with diminution of the sum of the orientational-exploratory activity parameters by 2.8 times ($p<0.05$) and the sum of all types of activities by 2.2 times ($p<0.05$) in comparison with primary values. In the group of atristamine-treated animals all parameters after TBI decreased also, but in any case this reduction did not reach a significant level.

Analysis of OFT parameters in the piracetam group before and after induction of TBI arouses the greater interest: the number of crossed squares decreased significantly by 3.8 times ($p<0.05$), number of explored holes – by 3.7 times ($p<0.05$), rearing – by 5.8 times ($p<0.05$). This correlates with diminution of the sum of orientational-exploratory activity – by 4.5 times ($p<0.01$) and the total sum of all types of activities – by 3.5 times ($p<0.01$).

Table 1. The impact of treatment with atristamine and piracetam on behavioral parameters of rats in open field test after TBI

Parameters (after 3 minutes)	Stage	Intact group, n=6	Disease group, n=7	Atristamine, 100 mg/kg, n=7	Piracetam, 400 mg/kg, n=6
Locomotor activity (crossed squares)	Before	13.7±6.4	16.6±5.4	21.3±3.2	15.3±4.6
	After	9.5±2.6	8.6±2.8	**13.7±3.7$^{\$}$**	**4.0±0.6$^{\&}$**
Orientational-exploratory activity					
– explored holes	Before	5.3±1.1	6.9±1.3	7.0±1.3	5.5±1.2
	After	2.8±0.7	**2.4±0.5$^{\&}$**	**4.7±0.6$^{\wedge/\$\$}$**	**1.5±0.6$^{\&}$**
– rearing	Before	6.3±1.5	6.3±1.9	7.3±1.7	5.8±1.7
	After	3.2±0.7	2.1±0.7	**4.0±0.5$^{\wedge/\$\$\$}$**	**1.0±0.4$^{*/\&}$**
– total	Before	11.7±2.6	13.1±3.1	14.3±2.6	11.3±2.7
	After	6.0±0.9	**4.6±1.2$^{\&}$**	**8.7±0.8$^{\wedge/\$\$\$}$**	**2.5±1.0$^{*/\&\&}$**
Emotional reactions					
– grooming	Before	1.5±0.3	1.4±0.6	1.7±0.5	2.3±0.6
	After	2.5±0.4	**1.1±0.3***	2.0±0.6	1.8±0.6
– defecation	Before	1.8±0.7	1.1±0.6	2.0±1,0	0.8±0.5
	After	1.2±0.7	0.1±0.1	1.7±0.9	0.3±0.3
– urination	Before	0.8±0.4	0.9±0.4	0.6±0.3	0.3±0.3
	After	0.5±0.3	0.4±0.3	0.7±0.3	0.0±0.0
– total	Before	4.2±1.1	3.4±1.1	4.3±1.1	3.5±1.4
	After	4.2±0.8	**1.7±0.4***	**4.4±1.1$^{\wedge}$**	2.2±0.8
Total sum of all types of activities	Before	29.5±7.9	33.1±7.8	39.9±5.3	30.2±6.5
	After	19.7±3.3	**14.9±3.0$^{\&}$**	**26.9±4.2$^{\wedge/\$\$}$**	**8.7±1.8$^{*/\&\&}$**

Notes. *&, && – p<0.05 and p<0.01 relatively initial parameters of this group using paired-samples t-test;*
** – p<0.05 relative to the intact group; ^ – p<0.05 relative to the disease group;*
$, $$ and $$$ – p<0.05, p<0.01 and p<0.001 relative to the piracetam group.

Analysis of intergroup differences in OFT after TBI showed that locomotor activity of the intact and the disease groups was on the same level. There was tendency to increase of this parameter in the group of atristamine-treated animals, which significantly exceeded it in the piracetam group by 3.4 times ($p<0.05$). The number of explored holes in the atristamine group was almost 2 times ($p<0.05$) higher compared to the disease group and 3.1 times ($p<0.01$) concerning parameter of the piracetam group. There are significant differences in rearing: the rate of the atristamine group exceeded this parameter of the disease group by 1.9 times ($p<0.05$) and of the piracetam group by 4 times ($p<0.001$). Obviously, the average value of the total sum of orientational-exploratory activity for all groups kept the aforementioned tendencies: a significant increase in the atristamine group by 1.9 times ($p<0.05$) compared to the disease group and by 3.5 times ($p<0.001$) relatively to the piracetam group was revealed.

A much smaller number of intergroup differences were observed in parameters of emotional reactions. A significant decrease of grooming (more than 2 times, $p<0.05$) and the total sum of emotional reactions (almost by 2.5 times, $p<0.05$) in the disease group compared to intact group has been noticed. At the same time, the total sum of emotional reactions in the atristamine group was higher compared to the disease group by 2.6 times ($p<0.05$). The total sum of all types of activities as an integral indicator completely reflects the overall impact of atristamine and piracetam on the conditions of the animals after TBI: after treatment with researched compound the sum was higher by 1.8 times ($p<0.05$) compared to the disease group and by 3.1 times ($p<0.01$) concerning rate of the piracetam group, which turned out to be 2.3 times ($p<0.05$) lower than intact control.

Elevated plus maze

The influence on the animals' anxiety level in EPM showed that concerning to the main parameter (time spent in the open arms of the maze) all groups of animals had relatively equal average values both in the primary state and after TBI (Table 2).

Generally, animals of the atristamine group after TBI were more active compared with other groups as noted by the number of animals that have made crossings (71.4%), which was significantly ($p<0.05$) different from the intact (0%) and the disease group (28.6%). This may indicates decrease of anxiety behavior in the overall level as well as stimulation of animals' research activity after TBI.

Table 2. The impact of treatment with atristamine and piracetam on anxiety in elevated plus maze test in rats after TBI

Parameters (after 5 minutes)	Stage	Intact group, n=6	Disease group, n=7	Atristamine, 100 mg/kg, n=7	Piracetam, 400 mg/kg, n=6
Latency, s	Before	16.0±4.2	44.0±21.3	13.1±6.6	15.0±5.3
	After	**37.7±5.7$^{\&}$**	29.6±12.5	**16.4±8.0***	31.8±13.2
Total time spent in open arms, s	Before	34.0±12.8	58.3±20.0	55.3±37.5	33.3±14.2
	After	37.7±5.7	51.6±14.1	46.4±14.8	46.5±12.9
Number of inter-arm crossings	Before	1.0±0.7	0.4±0.3	0.7±0.3	0.5±0.2
	After	0	0.4±0.3	1.7±0.9	0.3±0.2
Number of animals that have made crossings	Before	2/6	2/7	4/7	3/6
	After	0/6	2/7	**5/7^{F}**	2/6
Total time spent in "decision area", s	Before	20.8±4.1	58.3±20.0	17.1±7.3	16.8±4.7
	After	**37.7±5.7$^{\&}$**	33.9±11.7	33.7±14.3	32.2±13.0

Notes. *& – $p<0.05$ relatively initial parameters of this group using paired-samples t-test;*
** – $p<0.05$ relative to the intact group;*
F – $0.01<p<0.05$ relative to the intact and the disease groups using Fischer exact test.

Certain nuances appeared when analyzing parameters of latency and total time spent in the "decision area": in intact group they were significantly higher by 2.3 and 1.8 times, respectively. In this case all animals of this group did not make any crossings from one arm to another. This may be explained by the influence of ether anesthesia and the effect of "habituation" in the posterior study. In the atristamine group, duration of latency after TBI remained at the primary level, although it was significantly 2.3 times lower ($p<0.05$) relatively the intact group. However, in the disease group after TBI the trend towards decrease of both indicators was observed. Therefore, when using atristamine, indicators of animals were almost indistinguishable from initial state in contrast to the intact group, which may indicates the leveling of the negative impact of ether anesthesia.

Neurological deficit

McGraw scale in the modification of I.V. Gannushkina was used to assess neurological deficit of animals on the second and third day after brain injury [24]. This scale is usually used to assess disorders in more severe conditions (such as ischemic or hemorrhagic stroke) due to its insensitivity for mild injuries of the brain. But analysis of the results helps to detail the impact of atristamine and of piracetam on the severity of neurological deficit of rats after TBI and relates well with the results of other tests, thus application of the scale in this case is completely justified.

All animals from the disease group had certain manifestations of neurological deficit on the second day (tremor, lethargy movements, one-sided semiptosis, weakness of limbs). When converting specified symptoms into points (Table 3), we have reliable ($p<0.001$) difference from the intact group. The administration of atristamine significantly improved conditions of all animals on the second day as compared to the disease group ($p<0.001$), although some rats had weak manifestations of neurological deficit. Piracetam also improved conditions of animals relatively to untreated ($p<0.05$), but the score was significantly higher than indicators of uninjured ($p<0.01$) and of atristamine-treated rats ($p<0.01$).

Table 3. The impact of treatment with atristamine and piracetam on neurological deficit, depression level, cognitive functions, physical endurance, muscle tone and coordination of movements after TBI

Parameter	Stage	Intact group, n=6	Disease group, n=7	Atristamine, 100 mg/kg, n=7	Piracetam, 400 mg/kg, n=6
Neurological deficit (McGraw scale)					
Score	1st day	0	**1.000±0.109*****	**0.143±0.092**$^{\wedge\wedge\wedge/\$}$	**0.500±0.129**$^{**/\wedge}$
	2nd day	0	**0.571±0.071*****	**0**$^{\wedge\wedge\wedge/\$}$	**0.250±0.112**$^{*/\wedge}$
Vertical screen test					
Retention time, s	Before	126.2±29.7	176.9±3.1	158.0±21.7	130.7±31.4
	After	176.7±3.3	**125.9±15.2**$^{**/\&\&}$	**179.1±0.9**$^{\wedge\wedge}$	**166.3±6.4**$^{\wedge}$
Number of animals that held out all the time (3 minutes)	Before	3/6	6/7	5/7	4/6
	After	5/6	**1/7**^{F}	6/7	3/6
Forced swimming test					
Time of immobility, s	Before	90.0±9.2	80.4±8.2	78.3±9.4	76.2±8.7
	After	126.3±10.1	**156.9±12.7**$^{\&\&}$	**86.1±11.9**$^{*/\wedge\wedge/\$}$	**141.3±16.6**$^{\&\&}$
Extrapolation escape task					
Time for task implementation, s	Before	42.3±3.4	44.7±5.3	43.7±4.5	36.0±9.6
	After	77.7±33.2	67.0±29.4	47.6±23.7	93.7±33.7
Number of escaped animals	Before	6/6	7/7	7/7	6/6
	After	4/6	5/7	6/7	5/6
Weight-loaded forced swimming test					
Exhaustive swimming time, s	Before	248.2±48.4	242.1±37.1	195.3±22.9	289.8±55.3
	After	345.2±61.0	351.9±46.7	269.1±52.0	274.5±27.2

Notes. *& and && – $p<0.05$ and $p<0.01$ relatively initial parameters of this group using paired-samples t-test;*
****, **** *and* ********* *– $p<0.05$, $p<0.01$ and $p<0.001$ relative to the intact group;*
^, ^^ and ^^^ – $p<0.05$, $p<0.01$ and $p<0.001$ relative to the disease group;
$ – $p<0.05$ relative to the piracetam group; F – $p<0.01$ relatively initial parameters of this group, relative to the atristamine and the piracetam groups using Fischer exact test.

On the third day the severity of neurological deficit tended to weaken: none of the animals from the atristamine-treated group had manifestations of brain pathology, that when applying the scale significantly differed both from the disease group ($p<0.001$) and the piracetam group ($p<0.05$).

Vertical screen test

In the VST in the intact group of animals the posterior study showed a tendency to increasing retention time on the wire mesh screen (Table 3). Untreated animals as a result of trauma had an average retention time of 1.4 times ($p<0.01$) less as compared both to the initial parameters and to the intact group. In the atristamine group almost all the rats held out on experimental device total intended time (3 minutes) and by the parameters did not differ from the intact group. The administration of piracetam also increased the animals' capacity to climbing response compared to untreated animals by 1.3 times ($p<0.05$). It should be noted that the results correlate well with the assessment of neurological deficit by McGraw scale: piracetam reduces neurological symptoms and improves coordination, but yields to the effect of atristamine.

Forced swimming test

The results of the FST were of particular interest, because the antidepressant effect of atristamine in physiological state of animals has been proved in earlier studies [9, 10]. But the ability to affect the depression level under conditions of CNS pathology had not been studied yet.

In intact animals the trend to increase of average time of immobility after TBI for 40% compared with the initial indicator was noted (Table 3). This may be explained by the influence of ether anesthesia on the rats' CNS taking

into account monoaminergic theory of depressive disorders. In the group of untreated animals after TBI increasing of this parameter was much more marked – 95% ($p<0.01$). Piracetam did not affect the average time of immobility of animals: after trauma it increased by 85% ($p<0.01$) comparing with the initial state.

Special attention was attracted to the results of atristamine-treated animals – after they had a head injury average time of immobility remained at the initial level. The analysis of intergroup differences after TBI showed that the level of depression in this group was 1.5 times ($p<0.05$) lower than in the group of intact control and by 1.8 times ($p<0.01$) – compared to untreated animals. Thus, the administration of atristamine shows not only antidepressant effect in injured rats, but also attenuates the negative influence of ether anesthesia on neurotransmitter exchange in the brain.

Extrapolation escape task

Initial research in EET showed that the groups had almost the same initial parameters: all animals solved the task, and the average time practically did not differ (Table 3). After TBI in the intact group of rats there was a distinct tendency to increase of the average time spent for task solution (83%), but due to the large dispersion this growth did not reach the required significance level. In addition, objects that were not escaped (2 of 6) appeared in this group. This may be explained by the significant influence of ether anesthesia on cognitive function of animals. Logically, retesting rats should solve the escape task faster as a result of training. The same situation was observed in the disease group. Thus, mild TBI itself did not affect the ability of animals to perform the task. In the group administered with piracetam, this trend was more marked – the average time for task implementation increased by 160%. It is interesting, that in the atristamine group the increase of time was only 9%. Comparing with the results of the Porsolt test (FST) and EPM, it can testify in favor of protective effects against the negative influence of anesthesia on functions of the animals' CNS.

Weight-loaded forced swimming test

Analysis of the results showed that on the third day after mild TBI animals did not have any consequences that affect physical endurance (Table 3). It should be noted that in the intact group, the disease group and the group treated with atristamine there was the trend to increase the average time of swimming to 38-45%, which may be explained by the effect of "habituation". The average parameter in the group treated with piracetam remained at baseline, what in this case is atypical. Thus, the administration of atristamine does not improve physical endurance of animals after TBI, but the correspondence of the results with the intact group indicates the absence of negative effects.

Bilateral common carotid artery occlusion

Generalization of the results and eduction of neuroprotective properties of atristamine in the model of mild TBI gave grounds for continuing research on more severe models of brain lesions. For this purpose the model of brain ischemia caused by bilateral common carotid artery occlusion (BCCAO) in rats was chosen [25].

For express-analysis two groups of animals were formed: the first (n=7) – the group of disease control (untreated), the second (n=7) – administered with atristamine in dose 100 mg/kg (as stable suspension, intragastrically) once a day three times, the last time – 30 minutes before anesthetization. The procedure was performed under general barbamil anesthesia (60 mg/kg, intraperitoneal) using surgical approach: after isolation of the carotid arteries silk ligature immediately was overlapped upon them after separation of *nervus vagus* [25]. The animals were surveyed for five following days: mortality in each group was registered and neurological deficit of survived objects was assessed using McGraw scale.

The results disclosed that there were no significant differences in above mentioned parameters between the groups. For example, on the second day mortality both in the atristamine-treated group and the disease control group was 28.6% (2 animals from 7) with average neurological deficit scores 5.27±1.51 versus 3.21±1.76, respectively. In the following days of observation the same intergroup correlations retained.

Thus, overall analysis of the results shows that atristamine has effect neither on the mortality nor on the severity of neurological symptoms of survived animals with total ischemic brain damage.

CONCLUSION

The results of this research prove that treatment with atristamine (100 mg/kg) has protective effects against TBI. This has been demonstrated by reduced neurological deficit using McGraw scale, improved indicators of orientational-exploratory activities and emotional reactions in open field test, enhanced muscle tone and coordination of movements (vertical screen test), reduced level of anxiety (elevated plus maze). Also researched

substance had positive impact on cognitive functions (extrapolation escape task) and did not impair physical endurance (weight-loaded forced swimming test). According to this experimental model of pathology, the most definitely atristamine showed antidepressant activity (forced swimming test), confirming his competence as an antidepressant in pathological state. In addition, atristamine did not improve conditions of experimental animals in the model of bilateral common carotid artery occlusion, revealing certain "selectivity" of neuroprotective potential of the researched substance.

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