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EVALUATION OF THE EFFECT OF THE PIR-12 COMPOUND ON ANXIETY AND LOCOMOTOR ACTIVITY IN EXPERIMENTAL GLOBAL CEREBRAL ISCHEMIA IN RATS

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***Natalia Shabanova*[1], *Anastasia Gerashchenko*[1]✉, *Andrey Voronkov*[2]**

[1] *Pyatigorsk Medical and Pharmaceutical Institute — Branch of Volgograd State Medical University, Pyatigorsk, Russia*
[2] *The Volgograd State Medical University, Volgograd, Russia*

✉ *vahlushina@mail.ru*

ABSTRACT — A study was carried out to assess the effect of a new pyrimidine derivative (PIR-12 50 mg/kg) on anxiety and locomotor activity under conditions of global cerebral ischemia in rats. It was confirmed that the investigated compound PIR-12 helps to reduce the degree of anxiety, which is manifested in an increase in the indicators "Time at the central site" and "Time in the open arms of the maze" in 3,2 ($p<0,05$) and 3,1% ($p<0,05$). "Time in the closed arms of the labyrinth" and "the number of grooming acts" decreased against the background of the introduction of PIR-12 by 28,9% ($p<0,05$) and 4,1 times ($p<0,05$) compared with the control group animals, which surpasses the effect of the reference drug Cavinton. In addition, PIR-12 promotes an increase in the motor activity of ischemic rats: the number of hanging and standing rats was 7,4 times higher ($p<0,05$), 4,2 times ($p<0,05$), relative to the NC rat group, and the effect is comparable to the group of animals that received Cavinton.

KEYWORDS — cerebral ischemia, level of anxiety, locomotor activity, pyrimidine derivatives.

INTRODUCTION

Vascular diseases of the brain continue to be one of the most important problems in modern health care, since the incidence, disability and mortality from this nosology remain at a high level throughout the world [1]. Ischemia-induced disorders of cerebral circulation, pathophysiological and pathobiochemical processes in brain tissue, lead to a deficit of neurological and cognitive functions, which in turn correlate with the severity of brain damage [2]. In this connection, the need to include drugs with psycho- and neuroprotective effects in the therapy of acute cerebrovascular accidents is obvious and beyond doubt [3]. In experiments on rats, the positive effect of pyrimidine derivatives on neurological deficits resulting from impaired cerebral hemodynamics was proved [4], as a result of which it becomes expedient to further study pyrimidine derivatives as agents with neuroprotective effects.

Objective:

To evaluate the effect of the PIR-12 compound on anxiety and motor activity in experimental global ischemia of the rat brain.

MATERIALS AND METHODS

The study was conducted in accordance with the "Guidelines for Preclinical Trials of Drug Products" ed. by A.N. Mironov (a 2012 edition.) [5]. The animals were maintained in compliance with current best practices and standards of care in laboratory animals. The experiment was performed on 40 male Wistar rats m=220–240 g, divided into 4 groups (n=10). Rats were kept on a standard vivarium diet, with a natural succession of light and darkness. The first group was represented by falsely operated rats (FO), the second one — by negative control animals (NC). The both groups received an intraperitoneal suspension of Tween-80 in purified water. The third and fourth groups received reference drugs: Cavinton (3,2 mg/kg, LLC Gedeon Richter Pharma)[6]. The fourth group was administered the pyrimidine derivative PIR-12 (50 mg/kg), synthesized at the department of organic chemistry of the Pyatigorsk Medical and Pharmaceutical Institute (Pyatigorsk, Russia) — branch of Volgograd State Medical University[7]. All objects were injected intraperitoneally for ten days before the operation. In the second and subsequent groups global brain ischemia was simulated by bilateral occlusion of the common carotid arteries (under chloral hydrate anesthesia 350 mg/kg) [8, 9]. A day after the reproduction of ischemia, the behavioral activity of the animals was assessed in the "Elevated plus maze" (EPM) test. The Elevated plus maze is a behavioral test to study the activity, emotional state and level of anxiety in laboratory animals [10]. Animal behavior is recorded within 3 minutes. The indicators of anxiety were considered — the time spent on the central site (CS), the time in the open arms of the

maze (OA), the time in the enclosed arms of the maze (EA), the number of grooming acts. Changes in motor activity were judged by indicators — the number of transitions between arms (vertical activity), stance, hanging (horizontal activity). All findings were processed by means of variation statistics methods using the STATISTICA 6.0 software. The normality of distribution was assessed by the Shapiro-Wilk test. In the case of a normal distribution of the data, a parametric t-test was applied. In the case of abnormal distribution of the data, the statistical processing was performed using the Mann-Whitney U-test. The difference was considered significant at the significance level of more than 95% (p<0,05).

RESULTS

Under conditions of experimentally simulated cerebral ischemia in rats, an increase in the degree of anxiety was observed, which was manifested in a decrease in the time spent on the central site by 74,3% (p<0,05) and the time in the open arms of the maze by 71,2% (p <0,05), as well as an increase in the time in the enclosed arms of the maze by 55,4% (p<0,05) and the number of grooming acts by 3 times (p<0.05) compared with the initial indicators (CS — 28,4±1,8 sec., OA — 49,7±3,9 sec., EA — 101,9±5,3 sec., Grooming — 3,2±0,5 acts). Relative to the FO group of animals in negative control rats, the time in the center and in the open arms was significantly lower by 73,4% (p<0,05) and 72,3% (p<0,05), the time in the enclosed arms and grooming was higher by 57% (p<0,05) and 2,9 times (p<0,05), respectively. Under conditions of prophylactic administration of Cavinton, the time at the central site by 135,6% (p<0,05) and open sleeves was 93,7% (p<0,05) higher, the time in enclosed arms and grooming acts by 14,7% (p<0,05) and 3,4 times (p<0,05), respectively, were lower in relation to the analogous data of the NC group of animals, which may indicate a decrease in the level of anxiety in individuals. In the setting of intraperitoneal administration of the PIR-12 compound, the time in the center and in the open arms was 3,2 (p<0,05) and 3,1% (p<0,05) times higher, and the presence in the enclosed arms and the number of acts grooming is lower by 28,9% (p<0,05) and 4,1 times (p<0,05), respectively, compared with rats in the negative control group. In comparison with the group of rats treated with Cavinton, CS and OA in animals that were injected with PIR-12 was statistically significantly higher by 34,3% (p<0,05) and 59,6% (p<0,05), respectively. The time in the enclosed arms of the labyrinth, on the contrary, significantly decreased by 16,6% (p<0,05) in rats receiving PIR-12 relative to the reference drug Cavinton.

In addition to changes in the degree of anxiety in conditions of irreversible ligation of the carotid arteries, there is a decrease in horizontal and vertical motor activity. Thus, in animals of the NC group, a decrease in the number of transitions between the arms of the maze by 74% (p<0,05), hanging by 94% (p<0,05) and stands by 90,9% (p<0,05) compared with indicators before the reproduction of ischemia (transitions – 7,7±0,8, hanging — 8,4±1,1, stands — 14,3±1,8). In comparison with falsely operated rats, similar indicators in this group were also significantly lower: the number of transitions by 74,4% (p<0,05), hanging — by 94% (p<0,05), stands — by 90,6% (p<0,05). Against the background of taking the reference drug Cavinton, compared with untreated individuals, there was a significant change in the number of hanging (it was 6,6 times higher (p<0,05)) and stands (it was 2,7 times higher (p<0,05)) in there were no significant differences in the "number of transitions " indicator. The use of the test substance PIR-12 led to an increase in motor activity, so the number of hanging was 7,4 times higher (p<0,05), and the number of racks was 4,2 times higher (p<0,05) in relation to untreated rats. There were no statistically significant differences in the indicators characterizing the level of locomotor activity between the groups of rats treated with compound PIR-12 and the reference drug Cavinton.

CONCLUSION

In the experimentally simulated cerebrovascular insufficiency, the pyrimidine derivative under the laboratory code PIR-12 made it possible to reduce the degree of anxiety and increase the locomotor activity and showed an effect that was superior in strength to the comparison drug Cavinton.

REFERENCES

1. **Naderi Y, Panahi Y, Barreto GE, Sahebkar A.** Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review. // Neural regeneration research. – 2019. – Vol. 80. – N 8. – P. 773–782. DOI: 10.4103/1673-5374.268898.

2. **Shin TH, Lee DY, Basith S, Manavalan B, Paik MJ, Rybinnik I, Mouradian MM, Ahn JH, Lee G.** Metabolome Changes in Cerebral Ischemia. // Cells. – 2020. – Vol. 9. – N. 7. – P. 1630. DOI: 10.3390/cells9071630.

3. **Goenka L, Uppugunduri Satyanarayana CR, S SK, George M.** Neuroprotective agents in Acute Ischemic Stroke-A Reality Check. // Biomedicine & Pharmacotherapy. – 2019. – Vol. 109. – P. 2539–2547. DOI: 10.1016/j.biopha.2018.11.041.

4. **Shabanova N., Gerashchenko A., Voronkov A.** The effect of PIR-12 compound on survival and neurological deficits in experimental global cerebral

Table 1. *Assessment of the changes in the level of anxiety in the "Elevated plus maze" test against the background of the administration of the PIR-12 compound and Cavinton in experimental cerebral ischemia in rats*

Group	Time spent in the central square (sec.)	Time in the open arms of the labyrinth (sec.)	Time in the enclosed arms of the labyrinth (sec.)	Grooming (number of acts)
	M±m			
FO	27,4±1,8	51,7±4,4	100,9±4,1	3,3±0,5
NC	7,3±1,3#	14,3±1,4#	158,4±1,7#	9,5±1,3#
Cavinton	17,2±1,9*	27,7±3,8*	135,1±4,6*	2,8±0,5*
PIR-12	23,1±1,2*/Δ	44,2±4,5*/Δ	112,7±4,9*/Δ	2,3±0,6*/Δ

Note: *FO — false-operated rats; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-12 — a group of rats treated with PIR-12; # — statistically significant as compared to the FO rats (p<0,05); * — statistically significant as compared to the NC rats (p<0,05); Δ — statistically significant as compared to rats treated with Cavinton (p<0,05).*

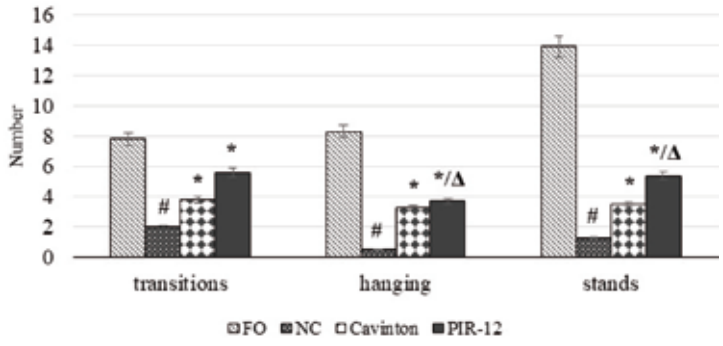

Fig. 1. *Assessment of locomotor activity in the Elevated plus maze" test against the background of the administration of the PIR-12 compound and Cavinton in experimental cerebral ischemia in rats*

Note: *FO — false-operated rats; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-12 — a group of rats treated with PIR-12; # — statistically significant as compared to the FO rats (p<0,05); * — statistically significant as compared to the NC rats (p<0,05);Δ — statistically significant as compared to rats treated with Cavinton (p<0,05).*

ischemia in rats // Archiv Euromedica. – 2020. – Vol.10. №. 4. – P. 26–28. DOI:10.35630/2199-885X/2020/10/4.5

5. **Mironov A.N.** The guidelines for preclinical studies of pharmaceuticals. Part one. – M.: Grif and K, 2012. – 944 p. (In Russ.)
6. **Nazarova L.E., Dyakova I.N.** Influence of ferulic acid on the necrosis zone resulting from occlusion of the middle cerebral artery //Medical Bulletin of Bashkortostan 2011. No. 3. P. 133–135. (In Russ.)
7. **Voronkov A.V., Shabanova N.B., Voronkova M.P., Lysenko T.A.** Study of cerebrotropic dose-dependent effect of pyrimidine derivative under pir-9 code against the background of experimental cerebral ischemia in rats. Pharmacy & Pharmacology. 2018;6(6):548–567. (In Russ.) DOI: 10.19163/2307-9266-2018-6-6-548-567
8. **Yamamoto M., Shima T., Uozumi T., Sogabe T., Yamada K., Kawasaki T.** A possible role of lipid peroxidation in cellular damages caused by cerebral ischemia and the protective effect of alpha-tocopherol administration // Stroke. – 1983. – Vol 14, N 6. – P. 977–982. DOI: 10.1161/01.STR.14.6.977
9. **Ghanbarabadi M., Falanji F., Rad A., Sharahi N.C., Amoueian S., Amin M., Molavi M., Amin B.** Neuroprotective effects of clavulanic acid following permanent bilateral common carotid artery occlusion in rats // Drug Development Research. – 2019. – Vol. 80. – N 8. – P. 1110–1119. DOI: 10.1002/ddr.21595
10. **Martynova O.V., ZHilinkova L.A., Gureev V.V., Martynov M.A., Beskhmel'nicyna, E.A., Kostina D.A., Anciferov O.V.SHkileva I.YU.** Issledovanie povedencheskih reakcij pri modelirovanii total'noj ishemii golovnogo mozga // Kubanskij nauchnyj medicinskij vestnik. – 2015. – N. 6. (In Russ.)