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COMPREHENSIVE ASSESSMENT OF NEUROLOGICAL DEFICIT AND ITS CORRECTION WITH PIR-10 COMPOUND IN EXPERIMENTAL FOCAL CEREBRAL ISCHEMIA OF RATS

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ABSTRACT — Introduction: Correction of motor dysfunction resulting from focal cerebral ischemia is one of the significant problems of pharmacology and experimental therapy. Pyrimidine derivatives in the experiment on rats previously showed cerebroprotective activity, as a result of which it was decided to test the PIR-10 compound for the ability to reduce neurological deficit. Methods: A study was conducted to assess the effect of pyrimidine derivative (PIR-10 50 mg/kg) on neurological deficiency in the conditions of focal cerebral ischemia in rats. The study used mature male Wistar rats weighing 220-240 g (n=40). Focal cerebral ischemia was reproduced by coagulation of the left middle cerebral artery (anesthesia chloralhydrate 350 mg/kg). Neurological deficits were assessed using the McGraw, Combs and D'alecy, Garcia scales. Results: The studied compound PIR-10 contributes to the reduction of neurological deficits in all selected schools in comparison with the control group of animals (McGraw by 68% ($p<0,05$), Combs and D'alecy by 4,3 times ($p<0,05$), Garcia by 84% ($p<0,05$)). In addition, the results obtained after administration of PIR-10 to rats showed a statistically significantly difference from the group of rats receiving a reference drug cavinton. However, complete recovery of motor functions after modeling focal cerebral ischemia was not observed in any group of rats. Conclusion: The PIR-10 compound is able to partially correct the neurological deficit that occurs in conditions of cerebral circulatory insufficiency, surpassing the activity of the reference drug cavinton.

KEYWORDS — focal cerebral ischemia, pyrimidine derivatives, neurological deficit.

INTRODUCTION

Focal cerebral ischemia occupies one of the leading places among the causes of disability due to the development of cognitive and neurological disorders [1, 2]. Motor dysfunction is the main cause of high disability in patients with impaired cerebral hemodynamics [3]. In this regard, compounds exhibiting cerebroprotective properties are of particular interest for the correction of emerging neurological deficits. In previous studies, the ability of pyrimidine derivatives to improve behavioral, mnestic and cognitive functions in the setting of global cerebral ischemia has been proven [4], which makes these compounds promising for further study for the ability to restore impaired musculoskeletal and neurological functions in cerebrovascular pathology.

Objective:

To assess neurological deficiency and correct it with PIR-10 compound in experimental global cerebral ischemia of rats

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=200–220 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 groups received reference drugs: Cavinton (3,2 mg/kg, LLC «Gedeon Richter Pharma) [6]. The fourth group was administered the pyrimidine derivative PIR-10 (50 mg/kg), synthesized at the department of organic chemistry of the Pyatigorsk Medical and Pharmaceutical Institute [7]. The second and subsequent groups modeled focal cerebral ischemia, by occlusion of the left middle cerebral artery (OLMCA) (under chloral hydrate anesthesia, 350 mg/kg) [8,9]. All objects were injected intraperitoneally immediately after the surgery and then once daily for three days. 24 hours after pathology modeling, neurological deficiency of animals according to the McGraw [10], Combs and D'alecy [11], Garcia [12] scales. The results were processed by the method of variational statistics using the STATISTICA 6.0 application program. The normality of

the distribution was evaluated by the Shapiro-Wilk criterion. In the case of normal data distribution, the parametric Student t-test was used. In the case of abnormal data distribution, statistical processing was carried out using the Man-Whitney U-test. Differences with a significance level of more than 95% ($p<0,05$) were considered reliable.

RESULTS

Focal cerebral ischemia in untreated animals contributed to inactivity, lethargy, half-ptosis and ptosis of the eyelids, paresis, and in some cases paralysis, in most cases the contralateral side of the injury. The neurological deficit of the NC group of rats reached 3,85 ± 0,21 points, which corresponds to the average severity of violations on the McGraw scale in the modification of Gannushkina (Fig. 1). Due to administration of the reference drug cavinton, rare cases of paresis were noted in animals, no limb paralysis was observed, the neurological status was 52% lower ($p<0,05$) relative to the animals of the negative control group. The introduction of the PIR-10 compound led to a significant decrease in the McGraw index compared with untreated animals and the group of rats treated with cavinton by 68% ($p<0,05$) and 32% ($p<0,05$), respectively.

Assessment of neurological deficits using the Combs and D'alecy scale enables to determine tenacity, balance and muscle strength in motor tests, which indicates the functional state of the extrapyramidal system. Coagulation of the left medial artery led to impaired motor skills and balance of the animals, which manifested itself in the inability to hold most of the muscles on a nylon rope, rod and screen-mesh. In the group of animals of negative control, a decrease in Combs and D'alecy scores was noted to 1,6±0,16, which was 79,7% ($p<0,05$) lower than in the group of rats (Fig. 2). Against the background of cavinton administration, motor function deficiency was 3,9 times ($p<0,05$) less pronounced in comparison with the group of rats without pharmacotherapy. Nevertheless, no complete recovery of motor disorders was observed. The introduction of the PIR-10 compound contributed to a decrease in motor deficit by 4,3 times ($p<0,05$) relative to untreated rats. In addition, the indicators of the groups of rats receiving PIR-10 and cavinton were statistically significantly different by 10,1% ($p<0,05$). Thus, in animals that were injected with PIR-10, the maximum improvement in motor functions was noted.

To assess the reaction of animals and the asymmetry of their movements, the Garsia neurological deficit score scale was used. OLMCA led to pronounced violations of asymmetry, motor skills and proprioception, manifested in a significant decrease in the neurological status in rats of the NC group on the Garsia scale by 2,8 times ($p<0,05$) relative to falsely operated individuals (17,7±0,15). The introduction of cavinton partially contributed to the improvement of motor functions (by 51% relative to untreated individuals). This indicator significantly differed from the values of the FO group, which indicates incomplete restoration of impaired functions. In the group of rats treated with PIR-10, the neurological index was 11,6±0,45, which was 84% ($p<0,05$) higher than in rats not treated with therapy, but 34% ($p<0,05$) lower than in the FO group. At the same time, despite the fact that this indicator exceeded that of Cavinton (by 22% ($p<0,05$)), a complete restoration of asymmetry was not observed.

CONCLUSION

The pyrimidine derivative under the laboratory code PIR-10 made it possible to partially correct the neurological deficit that occurs in conditions of cerebral circulatory insufficiency, and showed an effect in its strength superior to the reference drug cavinton.

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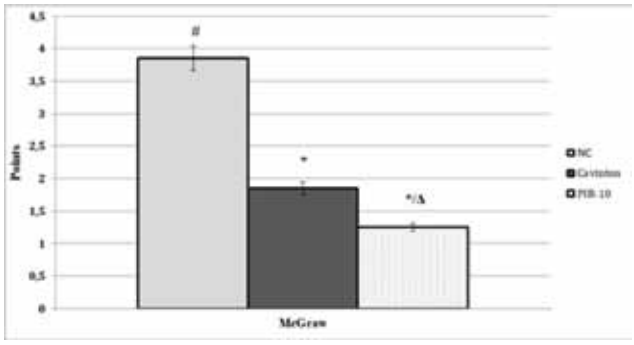

Fig. 1. *Assessment of neurological deficit on the McGraw scale while taking the studied compound and the reference drug in focal of cerebral ischemia*
Note: *FO — falsely operated rats ; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-10 — a group of rats treated with PIR-10; # — 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).*

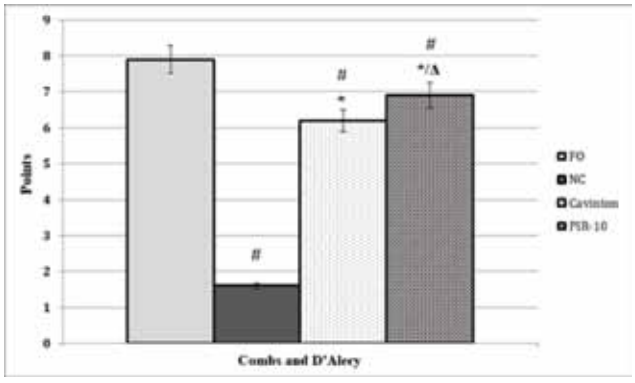

Fig. 2. *Assessment of neurological deficit on the Combs and D'alecy scale while taking the studied compound and the reference drug in focal of cerebral ischemia*
Note: *FO — falsely operated rats ; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-10 — a group of rats treated with PIR-10; # — 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).*

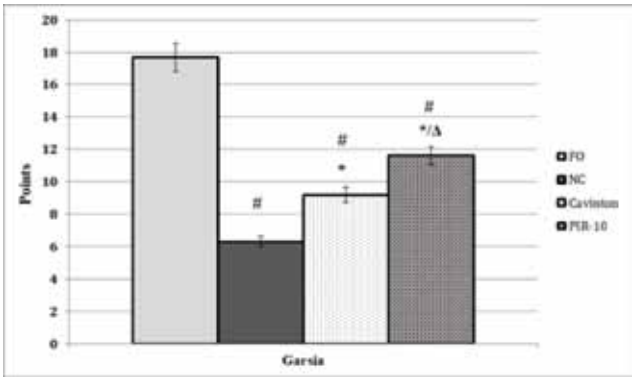

Fig. 3. *Assessment of neurological deficit on the Garsia scale while taking the studied compound and the reference drug in focal of cerebral ischemia*
Note: *FO — falsely operated rats; NC — negative control rats; Cavinton — a group rats treated with Cavinton; PIR-10 — a group of rats treated with PIR-10; # — 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).*

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