THE EFFECT OF PIR-4 SUBSTANCE ON THE NECROSIS ZONE IN EXPERIMENTAL FOCAL CEREBRAL ISCHEMIA IN RATS

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ABSTRACT — A study to assess the effect of a new pyrimidine derivative (PIR-4 at a dose of 50 mg/kg) on the size of the necrosis zone resulting from experimentally simulated focal cerebral ischemia in rats was carried out. It has been confirmed that the investigated substance PIR-4 contributes to a 16,48% reduction in the necrosis area ($p<0,05$) as compared to that in rats treated with a reference drug Cinnarizine (5,6 mg/kg); its potency is also comparable to that of Vinpocetine (3,2 mg/kg).

Introduction: Currently, there is an increase in the incidence of cerebral ischemia in the working-age population [1]. The development of new neuroprotective agents is a a high-priority task of modern Russian researchers. A potential cerebroprotective activity of pyrimidine derivatives [2] has been confirmed earlier; therefore the problem of the effect of these compounds on an ischemic zone (zone of necrosis) is of great interest.

Objective: To study the effect of PIR-4 substance on the necrosis zone in experimental focal cerebral ischemia in 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.) [3]. 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. Purified water with tween-80 was introduced to the first group rats (negative controls). The second and third groups received reference drugs: Cinnarizine (5,6 mg/kg) and Vinpocetine (3,2 mg/kg), respectively [4]. The fourth group received the investigational pyrimidine derivative PIR-4 (50 mg/kg) [5]. The model of focal cerebral ischemia was simulated by occlusion of the left middle cerebral artery (under chloral hydrate anesthesia, 350 mg/kg) [6]. All objects were injected intraperitoneally immediately after the surgery and then once daily for three days. The size of the necrosis zone was determined by means of the triphenyltetrazolium chloride method [7,8]. 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 and discussion: The simulated pathology led to the necrosis of 25,92±0,58% of the brain tissue in a group of animals who did not receive any pharmacological support (Fig. 1). Administration of Cinnarizine contributed to the reduction of the necrosis zone to 16,89±0,46 %, which is lower than that in untreated rats by 34,84%.($p<0,05$) The percentage of the necrotic brain tissue while using Vinpocetine was less than that in negative control rats by 44,06% ($p<0,05$). It must be noted that the necrosis zone in rats receiving Vinpocetine also differed significantly from that of rats treated with Cinnarizine (by 16,48%, $p<0,05$). The use of the experimental substance PIR-4 contributed to the reduction in the necrosis percentage to 13,51±0,65%, which significantly differed from the group of untreated rats and from animals receiving Cinnarizine injections (by 47,88% ($p<0,05$) and 20,01% ($p<0,05$), respectively). No significant differences between groups of animals treated with Vinpocetine and PIR-4 were detected.

Conclusion: In the experimentally simulated cerebrovascular insufficiency, a pyrimidine derivative (known under laboratory code PIR-4) reduced the percentage of necrotic brain tissue and showed the potency which was not inferior to that of the reference drug Vinpocetine and superior to that of Cinnarizine.

REFERENCES:

1. **Shmonin A. A.** Ligation of the middle cerebral artery in rats: comparison of models of focal cerebral ischemia//Regional circulation and microcirculation. 2011. No. 3. P. 68–76. (In Russ.)
2. **Voronkov A.V., Shabanova N.B., Kodonidi I.P., Shatalov I.S.** Cerebroprotective activity of new derivatives of pirimidine-4-(1H)-one PIR-9 and PIR-10 in irreversible occlusion of the common carotid artery. Pharmacy & Pharmacology. 2018;6(2):167–181. (In Russ.) DOI: 10.19163/2307-9266-2018-6-2-167-181

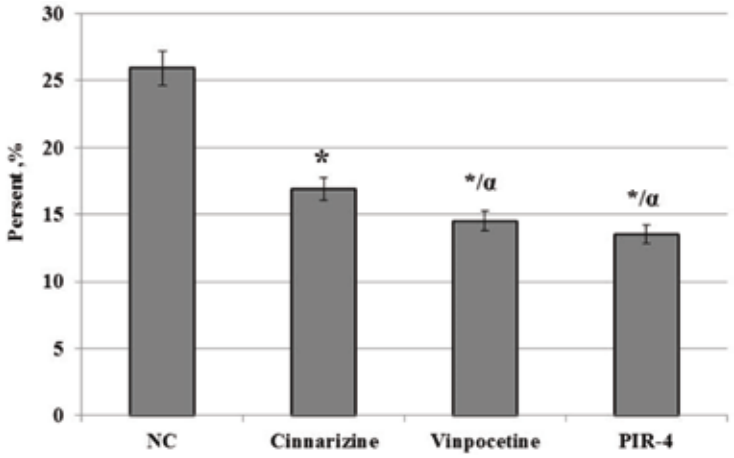

Figure 1. *Assessment of the effect of PIR-4 substance and the reference drugs on the necrosis zone under conditions of focal cerebral ischemia in rats*

Note: *NC — negative control rats; Cinnarizine — a group rats treated with Cinnarizine; Vinpocetine — a group of rats receiving Vinpocetine; PIR-4 — a group of rats treated with PIR-4; * — statistically significant as compared to the NC rats (p<0,05); a — statistically significant as compared to rats treated with Cinnarizine (p<0,05).*

3. **Mironov A. N.** The guidelines for preclinical studies of pharmaceuticals. Part one. – M.: Grif and K, 2012. – 944 p. (In Russ.)
4. **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.)
5. **Voronkov A.V., Shabanova N. B., Pozdnyakov D. I., Lugovoj I.S., Kodonidi I.P.** nfluence of new derivatives of pyrimidine-4(1H)-she's on the psycho-emotional imbalance and certain violations of energy metabolism in rats on the background of cerebral ischemia // Modern problems of science and education. – 2017– №5. (In Russ.)
6. Manual of stroke models in rats / ed. by Y. Wang-Fischer. – [S. l.] : CRC Press : Taylor& Francis Group, 2009. – XIII, 332 p.
7. **Bernauer, W.** Inhibiting effect of dexamethasone on evolving myocardial necrosis in coronary-ligated rats, with and without reperfusion/W. Bernauer// Pharmacology.- 1985. – Vol. 31, N 6. – P. 328–336.
8. **Gu M, Zheng A, Jin J, et al.** Cardioprotective Effects of Genistin in Rat Myocardial Ischemia-Reperfusion Injury Studies by Regulation of P2X7/NF-κB Pathway. Evidence-based Complementary and Alternative Medicine : eCAM. 2016;2016:5381290. doi:10.1155/2016/5381290.