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PRO/ANTIOXIDANT ACTIVITY OF A NEW PIR-10 SUBSTANCE (PYRIMIDINE DERIVATIVE) UNDER EXPERIMENTALLY SIMULATED FOCAL CEREBRAL ISCEMIA IN RATS

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The authors have performed a piece of research that focused on assessing anti-oxidant activity of PIR-10, a new pyrimidine derivative, as a risk factor causing disorders in the cerebral hemodynamics under experimentally simulated focal cerebral ischemia in rats. The experiment was accomplished on male Wistar rats with body weight equal to 220–240. 40 animals were distributed into 4 equal groups (n=10). The first group was made up of falsely operated animals; the second one was a negative control group; animals from both these groups were given a water suspension purified with TWEEN-80 in equivalent volumes. The third group included rats that were given a reference medication, namely Mexidol (emoxypine) (50 mg/kg). Animals from the fourth group were given an experimental substance with a laboratory cipher PIR-10 (50 mg/kg). All the examined objects were introduced intraperitoneally immediately after a surgery and during 3 days. Local cerebral ischemia was induced via coagulation of the left mesencephalic artery. All the manipulations with animals were performed under chloral hydrate narcosis (350 mg/kg). The performed research allowed to reveal that the given pathology caused an increase in lipid peroxidation products (diene conjugates (DC) and malonic dialdehyde (MDA)) with a simultaneous decrease in endogenous anti-oxidant protection (AOP) enzymes (superoxide dismutase, glutathione peroxidase, catalase). Mexidol application in a dose equal to 50 mg/kg allowed to correct these disorders due to an increase in antioxidant protection activity and a fall in concentrations of lipid peroxidation products. Introduction of the experimental substance PIR-10 also caused a decrease in DC and MDA concentrations but it didn't produce any effects on AOP system. Therefore, basing on the research results, we can assume that PIR-10 substance is a promising object for further research aimed at creating a medication with antioxidant properties that could allow to minimize epidemiologic risks related to cerebrovascular pathology.

Key words: *rats, cerebral ischemia, focal cerebral ischemia, antioxidant activity, lipid peroxidation, free radical oxidation, Mexidol, pyrimidine derivatives.*

Introduction. Ischemic cerebral affections occur due to various reasons, and a special role in the process belongs to lipid peroxidation activation and a sharp increase in intensity of free radical oxidation together with antioxidant protection enhancement and its subsequent decompensation [1-4]. A disorder in pro/antioxidant balance causes elevated risks of cerebral ischemia and aggravates its clinical course; dextrose metabolism deteriorates due to hyperproduction of free radicals and lactate-acidosis grows that results in damages to cellular membranes and, consequently, to cell death [5]. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GP) are basic enzymes participating in the endogenous antioxidant protection (AOP) as they help to level growing free radical oxidation processes thus reducing damages to the cerebral tissue [6]. Overall, these enzymes provide the so

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called "first line of defense" against reaction-active free oxygen forms [7]. Previously conducted experiments allowed to determine potential cerebral-protective effects produced by a new pyrimidine derivative with a laboratory cipher PIR-10 when global cerebral ischemia was simulated [8]. Experts have also examined influences exerted by some substances belonging to pyrimidine group on the AOP system [9, 10]. Hence we can assume that this derivative of pyrimidine-4(1H)-on has some antioxidation properties as a possible action mechanism that can help to considerably improve the epidemiologic situation as regards pathologies related to cerebral circulation disorders as well as substantially facilitate management of ischemic stroke risks.

Our research goal was to examine pro/antioxidant activity of a new substance PIR-10 (pyrimidine derivative) under experimentally simulated focal ischemia in rats.

Data and methods.

Animals. Laboratory rats were obtained from the vivarium of Pyatigorsk Medical and Pharmaceutical Institute, a branch of Volgograd State Medical University, The RF Public Healthcare Ministry. All the manipulations with animals were accomplished in full conformity with international standards of experimental ethics (The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, June 22, 1998)) and requirements fixed in Good laboratory practice (GLP). Animals were kept in makrolon cages with steel lattice covers and a hollow for feeding. Sawdust was used as litter. Rats were kept under controlled conditions similar to those in the vivarium, air humidity being equal to 65±5% and air temperature being 22±2ºC. The light was only natural, nutrition was standard, and all animals had free access to food and water. Water dishes, litter, and cages were replaced with new ones not less than once a week.

Experimental design. The experiment was performed on 40 male Wistar rats with body weight being equal to 220-240 gram. Animals were distributed into 4 equal groups (n = 10). The first group included falsely operated (FO) animals, the second group was negative control (NC). Animals from both groups were given a suspension of TWEEN-80 in purified water intraperitoneally. Animals from the third group were given a reference medication, Mexidol, in a dose equal to 50 mg/kg [11]. Animals from the fourth group were given an experimental substance, PIR-10, in a dose equal to 50 mg/kg [12]. The examined substance, the reference medication, and purified water with TWEEN-80 were introduced intraperitoneally just after ischemia had been simulated and further on during 3 days. Focal cerebral ischemia was simulated by left-side occlusion of the mesencephalic artery (via its coagulation). A cut was made on dehaired skin of an animal, below and 2 cm^2 to the right from the eye, muscles were separated from each other, and the process of the cheekbone was removed. A hole was made in the brainpan with a specially designed drill, and then the left mesencephalic artery was coagulated at the point where it crossed the olfactory tract with a coagulator which we had constructed ourselves [13]. All the manipulations were accomplished when animals were under chloral hydrate narcosis (350 mg/kg). When 3 days after the operation passed, animals were decapitated and their brains were quickly removed in order to obtain homogenate for further exploration. Cerebral homogenate was prepared on 100mM Tris-HCl buffer (with pH 7.4 in a ratio 1:10) [14].

Detectable parameters. We estimated diene conjugates (DC) concentrations in cerebral homogenate [basing on the conventional procedure by Z. Placer (1968) modified by V.BG. Gavrilov, M.I. Mishkorudnaya (1983).] [15], as well as TBA-active products concentrations recalculated as per malonic dialdehyde (MDA) [I.D. Stal'naya 1977] [16]. We determined activity of endogenous antioxidant protection enzymes in the post-nuclear fraction of cerebral homogenate including superoxide dismutase [V.N. Chumakov, 1977] [17], catalase [M.A. Korolyuk, 1988] [18], glutathione peroxidase [D.E . Paglia, 1967 modified by J.R. Prohaska, 1977] [19].

Statistical processing. All the obtained data were statistically processed with STATIS-

TICA 6.0 applied software package (StatSoft, Inc., the USA, compatible with Windows) and Microsoft Excel 2010. We determined a simple mean and its standard error ($M \pm m$) and applied Shapiro-Wilk test to assess whether distribution was normal. If data were distributed according to normal distribution law, we applied parametric Student's t-test. But if distribution wasn't normal, statistical processing was performed with Mann-Whitney U-test. If significance was higher than 95% (P<0.05), discrepancies were considered to be authentic.

Results and discussion. Concentrations of malonic dialdehyde and diene conjugates detected against cerebral ischemia were by 106.8% (P<0.05) and 280.6% (P<0.05) higher respectively in rats from the negative control group in comparison with falsely operated rats (Table 1). Simultaneously, concentrations of AOP enzymes in the negative control group went down in comparison with falsely operated rats; thus, SOD decreased by 25.2% (P<0.05); GP, by 49.9% (P<0.05); catalase, on the contrary, went up by 151.6% (P<0.05). This increase in catalase concentration can be caused by antioxidation protection system becoming more active as brain damage occurred. We should note that oxidants concentrations were extremely high as we can see from DC and MDA values, and SOD and GP were less active than usual [20]; it can be probably caused by an overall inability of AOP system to overcome hyperproduction of free radicals [21].

Table 1

Influence exerted by Mexidol and PIR-10 on malonic dialdehyde and diene conjugates concentrations in rats' brain homogenate against focal ischemia

Group	DC units of act./mg of protein)	MDA (units of act./mg of protein)
Falsely operated	13.68±0.51	7.03±0.09
Negative control	52.06±1.57#	14.54±0.33#
Mexidol	24.36±0.96*	8.79±0.2*
PIR-10	23.82±1.43*	9.27±0.68*

Note: # means the figure is authentic against falsely operated rats (P<0.05); * means the figure is authentic against rats from the negative control group (P<0.05).

Therapy with Mexidol led to a substantial decrease in DC (by 53.2%, P<0.05) and MDA (by 39.5%) in comparison with rats from the negative control group. Treatment with PIR-10 substance also resulted in lower concentrations of lipid peroxidation products; thus, DC went down by 54.2% (P<0.05), and MDA, by 36.2% (P<0.05) in comparison with rats from the negative control group. And we didn't detect any statistically significant discrepancies between rats who were given either Mexidol or PIR-10 substance.

As we can see from Figures 1 and 2, introduction of Mexidol led to greater activity of all antioxidant protection enzymes in comparison with the rats from the negative control group; thus, SOD activity increased by 20.4% (P<0.05); GP activity, by 34.0% (P<0.05); catalase activity, by 37.2% (P<0.05); and it is well in line with literature data [22]. But still, this increased superoxide dismutase activity and glutathione peroxidase activity was significantly lower than in falsely operated animals, by 9.9% and 33.0%, respectively.

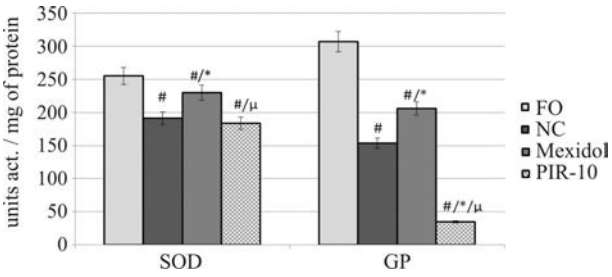

Figure 1. Influence exerted by Mexidol and PIR-10 on superoxide dismutase and glutathione peroxidase concentration in the post-nuclear fraction of rats' brain homogenate against focal ischemia

Note: # means the figure is authentic against falsely operated rats (P<0.05); * means the figure is authentic against rats from the negative control group (P<0.05); μ means the figure is authentic against rats that were given Mexidol (P<0.05).

Introduction of the examined substance PIR-10 led to a decrease in concentrations of endogenous AOP enzymes. Thus, SOD concentrations amounted to 183.46±2.88 units of act./mg of protein; it wasn't authentically different from the value detected for rats from the

negative control group and by 28.2% (P<0.05) and 20.2% (P<0.05) lower than in falsely operated rats and rats that were given Mexidol, respectively. Glutathione peroxidase in rats that were given PIR-10 went down authentically against animals from all other groups; against falsely operated rats, by 88.9% (P<0.05); against the negative control, by 77.6% (P<0.05); against rats that were given Mexidol, by 83.2% (P<0.05). Catalase was as active as in falsely operated rats and amounted to 0.33±0.01 nmol/min/mg of protein. This parameters was lower by 57.7% (P<0.05) than in rats from the negative control group, and by 69.2% (P<0.05) against rats that were given Mexidol.

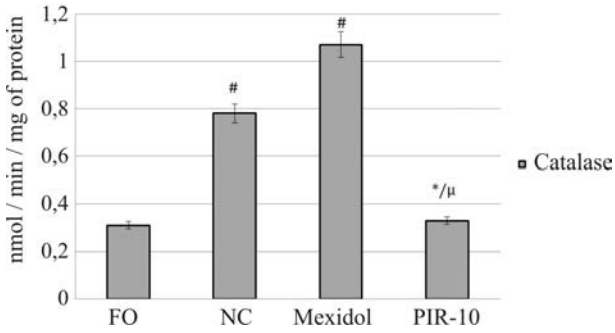

Figure 2. Influence exerted by Mexidol and PIR-10 on catalase activity in the post-nuclear fraction of rats' brain homogenate against focal ischemia.

Note: # means the figure is authentic against falsely operated rats (P<0.05); * means the figure is authentic against rats from the negative control group (P<0.05); μ means the figure is authentic against rats that were given Mexidol (P<0.05).

The obtained results can indicate that superoxide dismutase exerts its impacts on free oxygen forms thus transforming them into hydrogen peroxide [23], then glutathione peroxidase and catalase are likely to neutralize this hydrogen peroxide [24], and it can lead to lower concentrations of AOP enzymes against a decrease in formation of free radicals themselves. Analysis of experimental data is also confirmed by lower concentrations of lipid peroxidation products against introduction of PIR-10 substance. Given all the above-mentioned, we can conclude that the examined substance PIR-10 exerts its impacts on formation of free radicals but at the same time it doesn't activate the antioxidation system itself. And the reference substance Mexidol exerts its antioxidant impacts due to reinforcing antioxidation protection enzymes [25, 26].

Conclusions.

1. Experimentally simulated focal cerebral ischemia causes pro/antioxidant imbalance that is confirmed by an increase in concentrations of DC (by 280.6% (P<0.05)) and MDA (by 106.8% (P<0.05)), a decrease in concentrations of AOP enzymes (SOD by 25.2% (P<0.05), GP by 49.9% (P<0.05)) and an increase in catalase concentration 151.6% (P<0.05) in rats who didn't get any pharmaceutical support against falsely operated rats.

2. Introduction of Mexidol in a dose equal to 50 mg/kg results in activation of antioxidation protection system and it becomes obvious through a increase in SOD concentration by 20.4% (P<0.05), GP concentration by 34.0% (P<0.05), and catalase concentration by 37.2% (P<0.05), as well as through a decrease in concentrations of lipid peroxidation products (DC by 53.2% (P<0.05) and MDA by 39.5%) against animals from the negative control group.

3. Introduction of the examined substance PIR-10 leads to a decrease in concentrations of lipid peroxidation products (MDA by 36.2% (P<0.05), DC by 54.2% (P<0.05)) against rats from the negative control group but it doesn't cause any growth in concentrations of AOP enzymes. We can assume that this substance exerts antioxidation impacts as it reduces hyperproduction of free radicals without any effects produced on AOP enzymes themselves.

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Conflict of interests. The authors state there is no any conflict of interests.

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