STUDY OF THE NEW 4-PHENYLPYRROLIDINONE-2 DERIVATIVE PHARMACOKINETICS AND NEUROPROTECTIVE EFFECT IN THE ISCHEMIC STROKE ANIMAL MODEL

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The development of methods of drug therapy and rehabilitation in different periods of ischemic cerebral lesion is currently an urgent problem. Our study was aimed to investigate the pharmacokinetics and anti-ischemic effect of the new 4-phenylpyrrolidone-2 derivative in rats. To study the drug pharmacokinetics, the *Wistar* rats were once administered with the substance at a dose of 250 mg/kg, then, the substance distribution in blood and cerebral cortex was evaluated. Elimination half-life value was determined, which was 83.2 min. The substance remained in the brain tissue for 24 hours. To assess the anti-ischemic effect, the stroke was modeled by endovascular middle brain artery transition occlusion, and the drug was administered intravenously for 5 days at two doses, 250 and 125 mg/kg. After that the lesion focus volume was evaluated by MRI, as well as the neurological deficit severity, locomotor and explorative behavior. The studied drug significantly decreased the neurological deficit in model animals compared to control group (1.72 vs 4.4, $p < 0.05$). According to the MRI data, the effect on the ischemic focus was negligible, while the explorative behavior significantly increased under the influence of the 4-phenylpyrrolidone-2 derivative (hole board test, horizontal activity 12.1 ± 6.8, 22.5 ±10.5, $p < 0.05$). The data obtained allow us to conclude that the studied substance penetrates the blood-brain barrier (BBB), and accumulates in the brain tissue promoting the neurological deficit correction and increasing the explorative behavior in the ischemic stroke model animals.

Keywords: neuroprotective activity, pharmacokinetics, stroke, 4-phenylpyrrolidone-2, ischemic stroke models

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Compliance with ethical standards: the study was approved by the Animal Care and Use Committee of Pirogov Russian National Research Medical University (protocol № 48/2018 dated June 13, 2018). The animals were treated in strict compliance with the Declaration of Helsinki, Directive 2010/63/EU of the European Parliament and the Council (September 22, 2010) on the protection of animals used for scientific purposes, and Good Laboratory Practice guidelines established by the Order 708n of the Ministry of Healthcare of the Russian Federation (August 23, 2010).

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ИЗУЧЕНИЕ ФАРМАКОКИНЕТИКИ И НЕЙРОПРОТЕКТОРНОЙ АКТИВНОСТИ НОВОГО ПРОИЗВОДНОГО 4-ФЕНИЛПИРРОЛИДИНОНА-2 В МОДЕЛИ ИШЕМИЧЕСКОГО ИНСУЛЬТА НА ЖИВОТНЫХ

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Разработка методов медикаментозной терапии и реабилитации в разных периодах ишемического поражения головного мозга в настоящее время является актуальной проблемой. Целью исследования было изучить фармакокинетику и антиишемическое действие нового производного 4-фенилпирролидинона-2 на крысах. Для изучения фармакокинетики крысам линии *Wistar* однократно вводили вещество в дозе 250 мг/кг, затем оценивали его распределение в плазме и коре головного мозга. Установлен период полувыведения ($T_{1/2}$), 83,2 мин. Время нахождения вещества в тканях головного мозга составило 24 ч. Для оценки антиишемического действия проводили моделирование инсульта методом эндоваскулярной транзиторной окклюзии средней мозговой артерии, препарат вводили внутривенно в течение 5 дней в двух дозах, 250 и 125 мг/кг. Затем определяли размер очага поражения (с помощью МРТ), степень неврологического дефицита, локомоторную и исследовательскую активность. Исследуемое вещество значимо снижало неврологический дефицит у модельных животных по сравнению с контрольной группой (1,72 *vs* 4,4; $p < 0,05$). Влияние на очаг ишемии по МРТ было незначительным, а ориентировочно-исследовательское поведение под воздействием производного 4-фенилпирролидинона-2 значимо активизировалось («норковая камера», горизонтальная активность 12,1 ± 6,8, 22,5 ±10,5; $p < 0,05$). Полученные данные позволяют сделать вывод, что исследуемое вещество проходит через гематоэнцефалический барьер (ГЭБ), накапливается в коре головного мозга, способствуя коррекции неврологического дефицита и повышая исследовательскую активность у животных в модели ишемического инсульта.

Ключевые слова: нейропротекторная активность, фармакокинетика, инсульт, 4-фенилпирролидинон-2, модели ишемического инсульта

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Stroke is the second leading cause of death in Russia, by the number of cases it follows the myocardial infarction. According to the World Health Organization, 70–80% of stroke survivors become disabled [1–2]. Only thrombolytic therapy has proven pharmacological efficacy in the acute period of ischemic stroke, but it has a whole range of restrictions, the main of which is a narrow time interval. Only 5% of patients with acute ischemic stroke receive the thrombolytic therapy [1]. Rehabilitation therapy covers a much wider time interval, measured in weeks and months, so many researchers develop drugs in the context of rehabilitation medicine.

In recent years, both in Russia and abroad, a number of innovative solutions have appeared [3–5].

In the Department of Medical Chemistry and Toxicology of the Institute of Translational Medicine of Pirogov Russian National Research Medical University the new compound (laboratory number VRF_11) has been synthesized, which contains 4-phenylpyrrolidone-2 derivative as a pharmacophore [6]. According to *in silico* studies [7], the substance has an anti-ischemic, nootropic and cytoprotective potential.

The safety profile of the substance was established. To confirm the anti-ischemic effect of the new substance it was necessary to define the dose regimen and to estimate the main pharmacokinetic parameters.

The study was aimed to investigate the VRF_11 pharmacokinetics and to deduce the most effective dose regimen for the correction of neurological symptoms in the rat model of focal cerebral ischemia.

METHODS

The experiments were carried out on 85 adult male *Wistar* rats weighing 220 ± 12 g at the beginning of the study. The rats were received from the Stolbovaya nursery station. Animals were kept in the conventional vivarium of Pirogov Russian National Research Medical University with automatic change of day and night cycle (08:00–20:00, “day”, 20:00–08:00, “night”) and at least 12-fold change in the air volume of the room within an hour (temperature 20–24 °C, humidity 45–65%. Animals were fed with the "Chara" full-grain dry granular feed for laboratory animals (Assortiment-Agro; Russia; veterinary certificate form 3 250 № 3828680, declaration of conformity No. РОСС RU.ПН88.Д07428, valid until May 27, 2021) which was put *ad libitum* into the feeding recess of the steel grid cage lid. Animals were fed with water purified in accordance with the requirements of GOST 51232-98. Water in standard drinking bowls with steel nose caps was given *ad libitum*. For bedding the Rehofix corncob granules (JRS; Germany) were used.

Two series of experiments were conducted.

In the 1st series the VRF_11 pharmacokinetics was studied. VRF_11 was injected intravenously in the tail vein as the 200 mg/ml solution using the 0.4 × 8 mm needle (27G), in accordance with the rules of aseptic and antiseptic (at a dose of 250 mg/kg). Concentration of the substance in the blood plasma and brain tissue was determined at different time points. Blood sampling from the tail vein in a volume of 100–150 µl was performed after 15, 30, 60, 120, 240 min, 8, 12, 24 and 48 hours using the EDTA tubes. To study the VRF_11 accumulation in the brain, rats were euthanized in a CO2 box, after which the heart muscle was dissected, cannulas were inserted, and the carcass was washed with 1 liter of cold NaCl solution (0.9%). The brain was removed and the cerebral cortex was set apart. The fragment extracted was frozen and stored at –80 °C. Plasma was also frozen and stored at –30 °C.

In accordance with the recommendations on the study of pharmacokinetics [8] groups of animals were formed, 6 rats each (Table 1).

VRF_11 concentrations in the rats’ plasma and brain tissue samples were determined by high-performance liquid chromatography (HPLC). The SPD-20A absorbance detector (Shimadzu; Japan) was used to determine the VRF_11 concentration in the 10 µg/ml – 1 mg/ml range. The LC/MS 8030/8040 ultra fast triple quadrupole gas chromatograph mass spectrometer (Shimadzu; Japan) was used to determine the concentration of the substance in the 10 ng/ml – 10 µg/ml range. Calibration curves for the VRF_11 20–1000 µg/ml and 10 ng/ml – 50 µg/ml concentration ranges were plotted using the VRF_11 stock solutions and plasma of intact animals.

Determining the concentration of VRF_11 in the 10 µg/ml – 1 mg/ml range

To determine the drug concentration in the animals’ blood plasma, the following sample preparation routine was used. Acetonitrile in the amount of 300 µl containing 0.5% formic acid was added to 100 µl of blood plasma. After stirring and centrifuging (CM-50 centrifuge; ELMI; Latvia), 12,499 rpm for 3 min, supernatant was sampled and evaporated at the room temperature for 2 hours using the SpeedVac Savant SPD 1010 vacuum concentrator (Thermo Scientific; USA). The residue obtained was redissolved in 200 µl of the mobile phase. To prepare the mobile phase, 1.36 g of potassium dihydroorthophosphate (KH_2PO_4) was dissolved in 850 ml of deionized water. The 1.625 ml of diethylamine and 150 ml of acetonitrile were added to the resulting solution. Chromatographic conditions: isocratic elution mode; NUCLEODUR C_{18} ec 250/4.6 HPLC column (Macherey-Nagel; Germany), particle size 5 µm; column temperature 40 °C ± 0.1; eluent flow rate 0.8 ml/min; injection volume 10 µl; wavelength 220 nm.

Determining the concentration of VRF_11 in the 10 ng/ml – 10 µg/ml range

To determine the drug concentration in the animals’ blood plasma, the following sample preparation routine was used. Acetonitrile in the amount of 300 µl containing 0.5% formic

Table 1. VRF_11 pharmacokinetics study design (single intravenous administration)

Group №	Plasma sampling	Cerebral cortex
1	15 min; 240 min	240 min
2	60 min; 24 h	24 h
3	30 min; 8 h	8 h
4	48 h	
5	120 min; 12 h	
6		30 min
7		120 min

acid was added to 100 µl of blood plasma. After stirring and centrifuging (centrifugation conditions are similar to those described above), supernatant was evaporated using the vacuum concentrator (conditions described above) and redissolved in the 200 µl of eluent. Chromatographic conditions: Discovery® C18 HPLC Column (Supelco/Sigma-Aldrich; USA), particle size 5 µm, L × I.D. 15 cm × 4.6 mm; column temperature 40 °C ± 0.1; eluent flow rate 0.8 ml/min; injection volume 10 µl. Electrospray ionization (ESI) technique was used for analysis: desolvation line (DL) temperature 250 °C, heat block temperature 400 °C, nebulizer gas flow 2 l/min, drying gas flow 15 l/min, capillary voltage 4.5 kV, gas pressure for CID 60 kPa.

To determine the concentration of VRF_11 in brain tissue, 1.5 ml of acetonitrile containing 0.5% formic acid was added to 70-100 mg of animal brain, then, the brain was homogenized in a glass handheld homogenizer for 3 minutes. The resulting suspension was centrifuged twice (centrifugation conditions are similar to those described above). Supernatant was evaporated using the vacuum concentrator (conditions described above). A mixture of deionized water with acetonitrile (100:5) in the amount of 300 µl was added to the residue, then, the resulting mixture was stirred actively for 5 minutes. Centrifugation was carried out (conditions described above), and supernatant was analyzed. The mobile phase contained the mixture of 700 ml of deionized water, 3.5 ml of formic acid and 300 ml of acetonitrile. Chromatographic conditions: isocratic elution mode; Discovery® C18 HPLC Column (Supelco/Sigma-Aldrich; USA), particle size 5 µm, 150/4,6; column temperature 40 °C ± 0.1; eluent flow rate 0.8 ml/min; injection volume 10 µl. Electrospray ionization (ESI) technique was used: desolvation line (DL) temperature 250 °C, heat block temperature 400 °C, nebulizer gas flow 2 l/min, drying gas flow 15 l/min, capillary voltage 4.5 kV, gas pressure for CID 60 kPa. Processing and analysis of the results was performed using the LabSolution ver. 5.80 (Shimadzu; Japan) and Borgia 1.03 (Nauka Plus; Russia) software, as well as Microsoft Excel (Microsoft; USA) and Statistica 12 (Statsoft; USA) applications.

In a second series of experiments, cerebral infarction was modeled in rats to investigate the effect of VRF_11 administered at doses of 250 and 125 mg/kg on the neurological symptoms and behavior.

Experimental cerebral infarction was modeled by endovascular transient occlusion of the middle cerebral artery by the modified Koizumi method [9,10] followed by reperfusion. The duration of middle cerebral artery occlusion was 90 minutes.

The following groups of animals were formed, 8 rats each:

1. Control group. Animals were injected with saline 24 hours after reperfusion, once a day (5 days).

2. Treatment group. VRF_11 at a dose of 125 mg/kg was injected intravenously 24 hours after reperfusion, once a day (5 days).

3. Treatment group. VRF_11 at a dose of 250 mg/kg was injected intravenously 24 hours after reperfusion, once a day (5 days).

Laboratory animals magnetic resonance imaging (MRI) follow-up was performed using the ClinScan system (Bruker BioSpin; Germany) with 7 T magnetic field. To evaluate the cerebral infarction volume, the MRI scanning was performed on the 1st, 7th, 14th, 28th day after occlusion. The MRI protocol consisted of obtaining the T2-weighted images with the respiratory synchronization (TurboSpinEcho, Turbo Factor = 10, TR/TE = 5230/46 ms, voxel size 0.117*0.13*0.7 mm) in axial projection, starting from the 1st day after cerebral infarction modeling. The cerebral infarction volume follow-up was performed using the ImageJ software (Wayne Rasband; USA) analyzing the T2-weighted images. At the first stage, the area on each slice was measured, after which the total ficus volume was calculated by the formula $V = (S_1+\ldots+S_n)*(h + d)$, where S_1 was the 1st slice area, S_n was the area of slice n (mm^2), h was the slice thickness (mm), and d was the interslice gap (mm) [11].

Behavioral changes in rats were evaluated within 4 weeks after stroke modeling. The overall neurological functioning was assessed using the mNSS scale [12]. Motor deficit was evaluated using the following functional tests: the hole board test for rats (OpenScience; Russia) and the open field test (OpenScience; Russia). The hole board test was performed on the 10th and 24th day after stroke [13]. The open field test was performed on the 16th day. During the test the explorative and locomotor behavior was also assessed using the same parameters. In addition, the total path and speed of movement were calculated [14].

Statistical processing of the results was performed using the Statistica 12.0 software (Statsoft; USA). Mann–Whitney U-test, Student t-test for independent samples, as well as the descriptive methods with the definition of the arithmetic mean, standard deviation, standard error of the mean were used. The differences were considered significant at $p < 0.05$.

Table 2. VRF_11 blood level in laboratory animals after single intravenous administration at a dose of 250 mg/kg (n = 6, µg/ml)

Time point/№	1	2	3	4	5	6	M	SD
15 min	666.38	780.89	559.36	297.84	282.59	488.92	512.67	198.69
30 min	567.99	787.9	418.5	202.09	500.19	700.3	529.49	208.71
60 min	533.68	185.1	335.35	285.98	415.33	602.91	393.06	156.43
120 min	60.59	10.43	42.91	50.26	18.39	32.52	35.84	19.14

Note: M — mean value for the time point; SD — standard deviation.

Table 3. VRF_11 blood level in laboratory animals after intravenous administration at a dose of 250 mg/kg (n = 6, ng/ml)

Time point/№	1	2	3	4	5	6	M	SD
4 h	295.99	809.40	659.03	190.36	727.0	567.02	541.47	246.66
8 h	85.87	58.99	41.3	65.97	48.58	51.54	58.71	15.79
12 h	2883.98	1718.41	3074.25	1045.94	2120.61	1955.05	2133.05	753.03
24 h	147.47	126.16	114.34	93.21	105.18	97.93	114.05	20.18
48 h	73.37	62.76	56.88	46.37	52.32	48.72	56.74	10.04

Note: M — mean value for the time point; SD — standard deviation.

RESULTS

VRF_11 pharmacokinetics study (intravenous administration at a dose of 250 mg/kg)

Tables 2 and 3 contain the laboratory animals' VRF_11 blood concentration levels detected after the single intravenous administration. All the values fit the calibration curve.

The results of the experimental animals VRF_11 blood level measurement are presented in Fig. 1.

The data presented in Fig. 1, may be approximated satisfactorily by the single compartment model (without absorption), which is described by the following equation:

$$C = A \times \exp(-at),$$

where C is the studied drug level in the laboratory animals' blood, t is the time after the drug administration, A, a are the constants of a process describing a pharmacokinetic equation, which are associated with the constants describing the distribution of the studied drug in the body.

As a result of the pharmacokinetic data approximation using the Borgia 1.03 application, the following equation was obtained:

$$C = 580.143 \times \exp(-0.00833 \times t)$$

Table 4 demonstrates the indicators of the main pharmacokinetic parameters: C_{max}, T_{max}, $AUC_{(0\rightarrow\infty)}$, $T_{1/2}$, Cl, V_d.

Accumulation of VRF_11 in the cerebral cortex

Table 5 demonstrates the concentrations of VRF_11 in the cerebral cortex homogenate extracts after the single intravenous administration of the drug at a dose of 250 mg/kg. All the values fit the calibration curve.

Thus, VRF_11 penetrates the BBB, reaching the maximum concentration 30 minutes after administration. The drug is still detected in the rat cerebral cortex 24 hours after injection.

Monitoring the model of acute cerebral ischemia, dynamics of the infarction volume over time

The 3 groups of animals were studied, 8 rats each. One day after the modeling of cerebral infarction by the middle cerebral artery endovascular transient occlusion, the animals underwent MRI to control the volume of the lesion. Assessment of the ischemic lesion volume demonstrated that the modeling was performed correctly. Two rats with small size foci were excluded from the study (Table 5). In treatment group rats, after the MRI confirmation the studied drug was injected in the tail vein at doses of 125 and 250 mg/kg. The control animals were injected with the same volume of saline by the same method.

MRI follow-up results on the 1st, 7th, 14th and 28th day after reperfusion demonstrated that the studied substance at doses of 125 and 250 mg/kg had no effect on the average focus volume (compared to control group, which did not receive VRF_11) (Table 6). The infarction focus volume significantly decreased in all groups of animals by the end of the 1st month in relation to the 1st day after ischemia.

Neurological deficit assessment

Neurological deficit assessment using the mNSS scale was carried out on the 1st, 3rd and 5th day after reperfusion. On the 1st day the test was performed prior to the drug administration. According to the mNSS score, there were no significant differences between animals receiving VRF_11 at doses of 250 and 125 mg/kg and control animals (Table 6). As can be seen from the data presented (Table 7), when comparing neurological deficit scores on the 1st and 5th day, animals from the VRF_11 group showed statistically significant differences, whereas in control animals there were no such differences. It should be noted that there was a greater neurological deficit on the 1st day after reperfusion in the group receiving VRF_11 at a dose of 250 mg/kg compared to other groups, while an MRI scanning did not reveal differences in the ischemic foci volume in animals.

Behavioral response study

Behavioral study using the open field test, which was carried out on the 16th day after reperfusion, revealed significant differences in standing still and grooming duration, as well as in number of visits to the central zone in animals receiving VRF_11 at doses of 125 and 250 mg/kg compared to control group (Table 8).

In the hole board test 10 days after the ischemia modeling, significant differences were revealed between the control group and the group of animals injected intravenously with VRF_11

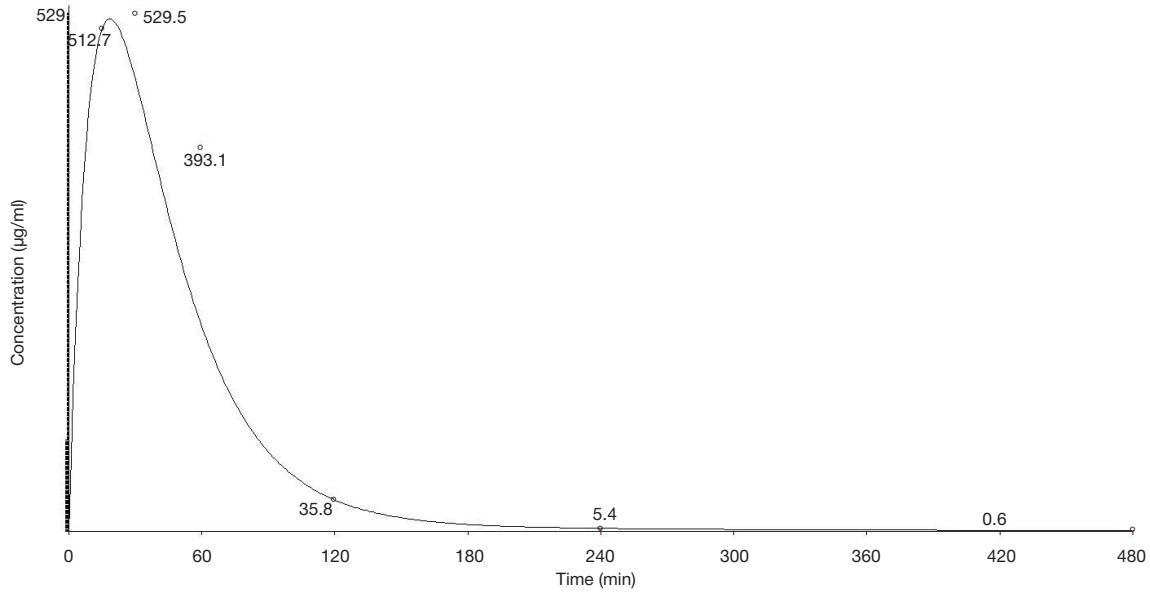

Fig. 1. VRF_11 kinetics in blood plasma after the single intravenous administration at a dose of 250 mg/kg

Table 4. Main pharmacokinetic parameters of VRF_11 (intravenous administration at a dose of 250 mg/kg)

Parameter	VRF_11
C_{max}, μg/ml	529
T_{max}, h	0.5
$T_{1/2res}$, min	83.2
$AUC_{(0\rightarrow\infty)}$, min × ng/ml	69645.1
Cl, ml/min	0.004
V_d, ml	0.862

(125 mg/kg and 250 mg/kg) (Fig. 2). There were differences in horizontal activity (sector crossing 12.1 ± 6.8, 22.5 ± 10.5 ($p < 0.05$) and 20.2 ± 14.7 ($p < 0.07$)) and vertical lactivity (number of stances 2 ± 1.6, 7.5 ± 4.0 ($p < 0.05$) and 7 ± 12.1 ($p < 0.07$)), standing still duration (46.6 ± 34.4, 1.0 ± 1.3 ($p < 0.01$) and 24.1 ± 21.9 ($p < 0.05$)) (control group, animals receiving VRF_11 at a dose of 125 mg/kg, and animals receiving VRF_11 at a dose of 250 mg/kg, respectively). On the 24th day after reperfusion in the hole board test, the experimental animals demonstrated more active explorative behavior compared to control group. Thus, significant различия ($p < 0.05$) differences were revealed in sector crossing (in animals receiving saline the value was 18 ± 7.3, in the group receiving VRF_11 at a dose of 125 mg/kg it was 31 ± 16.5 ($p < 0.03$), and in the group receiving VRF_11 at a dose of 250 mg/kg it was 26.3 ± 12.5 ($p < 0.05$)) and in the number of holes (5 ± 3, 10.5 ± 2.9 ($p < 0.01$) and 8.6 ± 2.9 ($p < 0.05$) respectively). The standing still duration significantly decreased only under the influence of VRF_11 at a dose of 125 mg/kg (22.9 ± 22.8 and 0.6 ± 2.3 ($p < 0.01$)).

DISCUSSION

Despite the widespread use of effective drugs for endovascular therapy, clinical outcomes after acute ischemic stroke still remain unsatisfactory [15]. Most patients retain motor impairment, their cognitive abilities decrease, and in many patients the psychoemotional sphere is impaired. As a result of neurological complications caused by stroke, neuroprotection and post-stroke rehabilitation has recently become an increasingly urgent problem [16].

The Department of Chemistry of Pirogov Russian National Research Medical University has an extensive experience of working with nootropic drugs [17, 18]. In this study, we used a promising substance, a derivative of 4-phenylpyrrolidinone-2, VRF_11, which, according to computer simulation, has neuroprotective properties.

The VRF_11 pharmacokinetics study results (Table 4) allow us to state that the drug penetrates the BBB and remains in the cerebral cortex during the day.

According to its pharmacokinetic parameters, VRF_11 is slightly different from the known nootropic drugs, such as piracetam and phenotropil. Thus, $T_{½}$ of piracetam administered intravenously is 4–5 hours [19], and $T_{½}$ of phenotropil is 2.77 hours [20]. The molecular weight of piracetam is 142 g/mol, and the molecular weight of phenotropil is 218 g/mol. A tendency to a decrease in half-life with an increase in molecular weight can be observed. Thus, in VRF_11 with molecular weight 252 g/mol the $T_{½}$ value is 1.26 hours.

VRF_11 remains in the brain tissue during the day. That is why we have chosen the dosage regimen once a day to avoid the effect of accumulation in the target organ. In the literature there is no clear opinion about the dosage regimen on metabotropic drugs (and on nootropic drugs in particular). Thus, according to official instructions, piracetam is taken up to 3 times a day, and phenotropil once per day. That is why the selection of the dosage regimen during the new compounds studies is one of the most difficult and time-consuming tasks. We have chosen the following algorithm: molecular weight conversion using the closest analogue (phenotropyl). The required dose of phenotropil is 100 mg/kg. After recalculation

Table 5. VRF_11 level (ng/g of tissue) in the cerebral cortex homogenate extracts after the single intravenous administration of the drug at a dose of 250 mg/kg

№ / Time, h	1	2	3	4	5	M	SD
0.5	1813.89	1167.11	2129.94	1566.63	2058.27	1747.17	392.69
2	354.12	396.38	363.63	407.07	380.50	380.34	22.01
4	260.34	233.58	289.16	218.68	250.97	250.54	26.88
8	144.54	158.01	171.49	126.96	140.43	148.29	17.05
24	20.63	23.0	18.34	17.37	18.84	19.64	2.22

Note: M — mean value for the time point; SD — standard deviation.

Table 6. Ischemia focus volume, mm^3 (M ± *m*; *n* = 8)

Day of scanning	Control (saline)		VRF_11, 125 mg/kg		VRF_11, 250 mg/kg	
	M	SD	M	SD	M	SD
1	131.14	24.60	133.25	33.16	133.66	23.72
7	111.79	24.04	107.53	33.38	115.91	20.75
14	100.43	20.63	93.64*	29.82	106.86	21.87
28	83.04*	15.63	81.6*	26.97	91.16*	20.67

Note: M — mean value for the time point; SD — standard deviation; *— $p < 0.05$ (comparison of mean values within the same group in relation to the 1st day).

Table 7. Neurological deficit assessment using the mNSS scale (M ± *m*; *n* = 8)

	Control		VRF_11 125 mg/kg		VRF_11 250 mg/kg	
	Mean	SD	Mean	SD	Mean	SD
1st day	5.43	3.55	6.8	3.6	9.1	2.75
3rd day	4.43	2.63	3.8	2.48	7.1	2.39
5th day	3.71	2.81	2.4*	1.52	6.5*	2.14

Note: * — $p < 0.05$ (comparison of mean values within the same group in relation to the 1st day).

Table 8. Activity indicators in the open field test on the 16th day after reperfusion (M ± *m*; *n* = 8)

	Control		VRF_11 125 mg/kg		VRF_11 250 mg/kg	
	Mean	SD	Mean	SD	Mean	SD
Sector crossing	35.83	9.133	40.87	12.04	42.33*	14.95
Center	0.16	0.4	1.13*	1.13	1.33*	1.75
Standing still, duration	36.83	33.73	9.37*	6.948	21.67	23.09
Grooming, duration	29.167	22.75	10.62*	13.23	17.33	14.08

Note: * — $p < 0.05$ (comparison of mean values in relation to the control group).

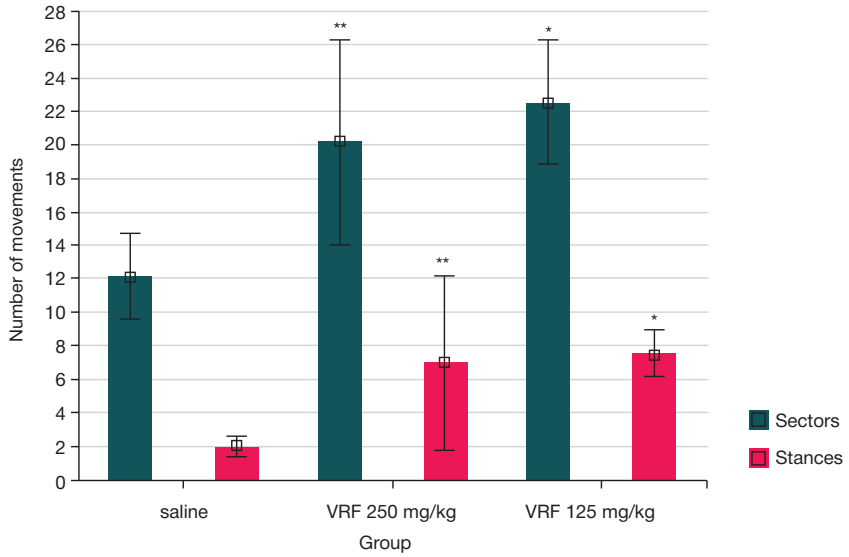

Fig. 2. * — $p < 0.05$ (comparison of mean values in relation to the control group); ** — $p < 0.07$ (there is a tendency to significant differences when comparing average values in relation to the control group). Animal behavior during the hole board test on the 10th day after ischemia modeling

taking into account the molecular weight, the dose of the studied substance was 125 mg/kg. In order to reveal the possible dose dependent effects, a dose 2 times higher than the calculated was used in the study (250 mg/kg).

The dose of 125 mg/kg was more effective for correction of ischemic lesion than the dose of 250 mg/kg, as evidenced by a greater neurological deficit decrease, as well as the explorative behavior increase during the hole board test and the open field test. According to literary sources, the effect of piracetam, on the contrary, is achieved only at high concentrations [21]. Our observations suggest the feasibility of studying a larger range of doses, since different doses of the drug can not only affect its effectiveness, but also lead to opposite pharmacological effects [20].

According to MRI scanning results, VRF_11 does not affect the volume of the lesion focus, but significantly reduces neurological symptoms in rats. This observation limits the assessment of the test compound as a neuroprotector, but refers to the possibility of its use among other rehabilitation drugs. The observation is interesting in terms of studying the possible mechanisms of VRF_11 effect. Certainly, it will be the base of our future studies aimed to find a specific target for the drug.

CONCLUSIONS

Calculation of the new 4-phenylpyrrolidone-2 derivative (laboratory number VRF_11) main pharmacokinetic parameters allowed us to come to the following conclusions: 1. VRF_11 passes through the blood-brain barrier (BBB) and accumulates in the brain tissue. 2. Maximum concentration of VRF_11 in the studied time range is achieved 0.5 h after the substance administration. 3. After 24 hours, VRF_11 in the cerebral cortex is on the verge of detection. 4. VRF_11 at a dose of 125 mg/kg significantly corrects the neurological functions impairment resulting from modeling of focal ischemia in rats.

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