



HEMORHEOLOGICAL PROPERTIES OF THE 5-HT_{2A}-ANTAGONIST OF THE 2-METHOXYPHENYL-IMIDAZOBENZIMIDAZOLE DERIVATIVE OF THE RU-31 COMPOUND AND CYPROHEPTADINE, IN COMPARISON WITH PENTHOXYPHYLLINE

D.S. Yakovlev^{1,2}, L.V. Naumenko¹, K.T. Sultanova^{1,2}, A.A. Spasov^{1,2}

¹ Volgograd State Medical University

1, Pavshikh Boytsov Sq., Volgograd, Russia, 400131

² Volgograd Medical Research Center

1, Pavshikh Boytsov Sq., Volgograd, Russia, 400131

E-mail: sultanova.pharma@gmail.com

Received 31 Jul 2020

Accepted 15 Oct 2020

Migraine and its comorbid conditions are pathogenetically associated with many factors, including hemorheological disorders. A class of drugs with a 5-HT_{2A} antagonistic mechanism of action, is promising for the prevention and treatment of migraine attacks and concomitant pathologies.

The aim of the research is to study and compare a hemorheological activity of anti-migraine drugs, antagonists of 5-HT_{2A} receptors of cyproheptadine, and a new drug that completed preclinical studies of the 1-(2-diethylaminoethyl)-2-(4-methoxyphenyl)-imidazo[1,2-a]benzimidazole derivative of the RU-31 compound.

Materials and methods. The study of the hemorheological activity of the RU-31 compound and cyproheptadine, was carried out using an experimental model of rabbit blood hyperthermia *in vitro*. Pentoxifylline was used as a reference drug. In the course of the work, the parameters of blood viscosity, aggregation and deformability of erythrocytes were recorded.

Results. It has been established that in the concentration of 1 μ M, the RU-31 compounds reduce blood viscosity by 17% at high shear rates, which is comparable with pentoxifylline in the concentration of 100 μ M on the activity level. In the concentration of 1 μ M, cyproheptadine also causes a general tendency to reduce blood viscosity at high shear rates, being inferior in activity to the RU-31 compound and pentoxifylline.

In the concentration of 1 μ M, the RU-31 compound has a pronounced effect on the aggregation ability of erythrocytes in autologous plasma, reducing the aggregation rate by 70%, while the level of activity is not inferior to the drug compared to pentoxifylline in the concentration of 100 μ M, and surpasses the drug cyproheptadine. For the RU-31 compound and cyproheptadine, no significant effect on the deformability of erythrocytes has been shown.

Conclusion. The capacity of cyproheptadine and the RU-31 compound to influence the rheological properties of blood by reducing blood viscosity and aggregation of erythrocytes has been revealed.

Keywords: migraine; 5-HT_{2A}-antagonists; hemorheology; microcirculation; deformability; aggregation

ГЕМОРЕОЛОГИЧЕСКИЕ СВОЙСТВА 5-НТ_{2A}-АНТАГОНИСТА ПРОИЗВОДНОГО 2-МЕТОКСИФЕНИЛ-ИМИДАЗОБЕНЗИМИДАЗОЛА СОЕДИНЕНИЯ РУ-31 И ЦИПРОГЕПТАДИНА В СРАВНЕНИИ С ПЕНТОКСИФИЛЛИНОМ

Д.С. Яковлев^{1,2}, Л.В. Науменко¹, К.Т. Султанова^{1,2}, А.А. Спасов^{1,2}

¹ Федеральное государственное бюджетное образовательное учреждение

высшего профессионального образования «Волгоградский государственный медицинский университет»

Министерства здравоохранения Российской Федерации

400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1

² Государственное бюджетное учреждение «Волгоградский медицинский научный центр»

400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1

E-mail: sultanova.pharma@gmail.com

Получено 31.08.2020

Принята к печати 15.10.2020

For citation: D.S. Yakovlev, L.V. Naumenko, K.T. Sultanova, A.A. Spasov. Hemorheological properties of the 5-HT_{2A}-antagonist of the 2-methoxyphenyl-imidazobenzimidazole derivative of the RU-31 compound and cyproheptadine, in comparison with pentoxifylline. *Pharmacy & Pharmacology*. 2020;8(5):345-353. DOI: 10.19163/2307-9266-2020-8-5-345-353

© Д.С. Яковлев, Л.В. Науменко, К.Т. Султанова, А.А. Спасов, 2020

Для цитирования: Д.С. Яковлев, Л.В. Науменко, К.Т. Султанова, А.А. Спасов. Гемореологические свойства 5-НТ_{2A}-антагониста производного 2-метоксифенил-имидазобензимидазола соединения РУ-31 и ципрогептадина в сравнении с пентоксифиллином. *Фармация и фармакология*. 2020;8(5):345-353. DOI: 10.19163/2307-9266-2020-8-5-345-353

Мигрень и ее коморбидные состояния патогенетически ассоциированы со многими факторами, в том числе и с гемореологическими нарушениями. Перспективным в отношении профилактики и лечения мигренозных атак и сопутствующих патологий является класс препаратов с 5-HT_{2A} -антагонистическим механизмом действия.

Цель. Изучение и сравнение гемореологической активности противомигренозных средств антагонистов 5-HT_{2A} -рецепторов ципрогептадина и нового средства, завершившего доклинические исследования производного 1-(2-диэтиламиноэтил)-2-(4-метоксифенил)-имидазо[1,2-а]бензимидазола соединения РУ-31.

Материалы и методы. Изучение гемореологической активности соединения РУ-31 и ципрогептадина проводили с использованием экспериментальной модели гипертермии крови кроликов *in vitro*. В качестве препарата сравнения использовался пентоксифиллин. В ходе работы регистрировались параметры вязкости крови, агрегации и деформируемости эритроцитов.

Результаты. Установлено, что соединение РУ-31 в концентрации 1 мкМ снижает вязкость крови на 17% при высоких скоростях сдвига, что по уровню активности сопоставимо с пентоксифиллином в концентрации 100 мкМ. Ципрогептадин в концентрации 1 мкМ также вызывает общую тенденцию снижения вязкости крови при высоких скоростях сдвига, уступая по активности соединению РУ-31 и пентоксифиллину. Соединение РУ-31 в концентрации 1 мкМ оказывает выраженное действие на агрегационную способность эритроцитов в аутологичной плазме, снижая показатель агрегации на 70%, при этом, по уровню активности, не уступая препарату сравнения пентоксифиллину в концентрации 100 мкМ и превосходя препарат ципрогептадин. Для соединения РУ-31 и ципрогептадина не показано значимого влияния на деформируемость эритроцитов.

Заключение. Выявлена способность ципрогептадина и соединения РУ-31 оказывать влияние на реологические свойства крови путём снижения вязкости крови и агрегации эритроцитов.

Ключевые слова: мигрень; 5-HT_{2A} -антагонисты; гемореология; микроциркуляция; деформируемость; агрегация

INTRODUCTION

Migraine is a common disease that is associated with an impaired blood supply to the brain. Migraine often accompanies the development of disabling complications. For this disease, the presence of concomitant disorders, such as arterial hypertension, ischemic heart disease, myocardial infarction, stroke, is possible [1, 2]. The associative links between these pathological conditions are confirmed by the data obtained in a number of epidemiological, clinical, as well as experimental studies of their pathological physiology. Risk factors (smoking, the female sex, hormonal contraceptives, a high frequency of migraine attacks and symptoms of aura) significantly increase the risk of these comorbid conditions, including ischemic stroke [3]. It is known that the risk of ischemic stroke increases significantly the presence of migraine pathology in patients, especially accompanied by aura. Neurovascular changes that occur during migraine attacks, are often accompanied by the increased blood viscosity, which mediates an even greater deterioration of the blood flow in the system of small vessels, and aggravates pathological processes [4, 5].

Several hypotheses explain the increased risk of ischemic stroke in the people with migraine. First, during the development of a migraine attack, there are neurovascular changes that can mediate the onset of a stroke. Second, these links are based on common pathophysiological aspects. Thus, the stroke can be caused by pronounced hemorheological changes associated with hypercoagulation and increased blood viscosity (with low hematocrit and increased aggregation of erythrocytes), predisposing to the development of thrombosis in the cerebral arteries and the occurrence of "rheological stroke" [6, 7].

It is known that blood is a non-Newtonian fluid (its

viscosity depends on the shear rate) and exhibits shear thinning (decrease in viscosity is accompanied by increase in the rate or shear stress), which determines the features of its effect on various parts of the vascular bed. It is customary to distinguish rheological parameters (viscosity of the whole blood, deformability and aggregation of erythrocytes) that determine the blood flow in the large vessels with a diameter of more than 200 μm , and in the microvasculature (less than 200 μm) [8].

It has been registered that in small arteries, arterioles and venules, a 75% decrease in blood viscosity is determined by the processes of erythrocyte aggregation, and a 25% decrease – by cell deformability. At the same time, in exchange capillaries, the deformability of erythrocytes is an important microrheological parameter that ensures the efficient passage through the vessels, the diameter of which is smaller than the size of the cells, which is ensured by the membrane deformability.

Blood realizes its functions in the body through its fluidity property; its violation is manifested in an increase in blood viscosity, which is formed as a result of unidirectional shears in rheological parameters, including an increase in plasma viscosity, an increase in erythrocyte aggregation and a decrease in the deformability of their membranes. A "high blood viscosity syndrome" develops; it triggers a number of adverse hemodynamic consequences, such as slowing down a blood flow, increasing a total peripheral vascular resistance, increasing blood pressure and blood deposition in the venous bed. These processes lead to a violation of microcirculation and decreasing in the oxygen delivery to tissues and organs – and ischemia is developing. The development of a "high blood viscosity syndrome" and a serious impairment of rheological properties associated with it,

are observed in many diseases, including the development of ischemic stroke in transient migraine disorders. Based on this, the problem of the migraine treatment, as well as the prevention of its comorbid conditions, remains relevant [9].

A subclass of anti-migraine drugs with a 5-HT₂-blocking mechanism of action is promising for the prevention of concomitant disorders in migraine attacks [10, 11]. In earlier preclinical studies (Project No.14.N08.11.0159), a new derivative of 2-methoxyphenyl-imidazobenzimidazole – 5-HT_{2A} antagonist – RU-31 was identified; it demonstrates anti-migraine properties and is positioned not only for migraine headaches treatment, but also for the attacks prevention [12, 13]. Activation of type 2 serotonin receptors causes constriction of cranial vessels, increases capillary permeability and alters platelet aggregation. It is believed that blockers of this receptor subtype, are capable of affecting cerebral microcirculation [14], and are also capable of inhibiting platelet and erythrocyte aggregation, thereby exerting a positive effect on the rheological properties of blood, improving microcirculation [15–17].

Thus, it is important to study 5-HT_{2A} antagonists as biologically active compounds capable of not only providing an anti-migraine effect, but also influencing the rheological characteristics of blood.

THE AIM of the research is to study and compare a hemorheological activity of anti-migraine drugs, antagonists of 5-HT_{2A} receptors of cyproheptadine, and RU-31.

MATERIALS AND METHODS

Experimental animals

The experiments were performed on the blood samples taken from sexually mature male Chinchilla rabbits, in the amount of 6 animals (Breeding nursery of laboratory animals “Krolinfo” Ltd, Moscow region), weighing 4.0–4.4 kg. Before the start of the study, all the rabbits were adapted for 14 days, kept single in cages in the vivarium of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, the Ministry of Health of Russia. The clinical condition of the animals was monitored daily by visual inspection. The animals with abnormalities found out during the examination, were not included in the experimental studies.

The animals were kept in standard conditions in accordance with the Decree of 29 August, 2014 No.51 “On the approval of SP 2.2.1.3218-14” Sanitary and epidemiological requirements for the design, equipment and maintenance of experimental biological clinics (vivaria)”, as well as in accordance with the directive of the European Parliament and the Council of the European Union 2010/63/EC dated 22 September, 2010 “On the protection of animals used for scientific purposes”.

The animals were kept in controlled environmental conditions with the air temperature in the range of 20–22°C and the relative humidity of 30–70%. In the rooms for the animals, a twelve-hour lighting cycle was main-

tained, with a combined type of lighting – natural and fluorescent – during the daylight hours. The rabbits were kept in standard laboratory cages for large rodents with sawdust bedding and an access to drinking bowls and feeders *ad libitum*.

The study was complied with the ethical standards of the responsible committee of human experimentation (institutional and national) and the Declaration of Helsinki. The Regional Research Ethics Committee of the Volgograd Region (registration number IRB 00005839 IORG 0004900 (OHRP)) approved of the conduct of this experimental study – protocol No. 2032-2017 dated 26 June, 2017.

Investigated substances

The chemical substance RU-31 (1-(2-diethylaminoethyl)-2-(4-methoxyphenyl)imidazo[1,2-a]benzimidazole) [18], was synthesized at the Scientific Research Institute of Physical and Organic Chemistry of the Southern Federal University (Rostov-on-Don).

RU-31 was investigated in the concentration of 1 μM (an equimolar concentration to cyproheptadine with a serotonin-blocking effect [19, 20]). Testing was performed on each blood sample from every rabbit. The compounds were directly incubated *in vitro*.

Reference drugs

5-HT₂ antagonist – cyproheptadine hydrochloride (Merck, USA) and a microcirculation corrector – pentoxifylline (Merck, USA) were used as reference drugs.

For pentoxifylline, an effect on the rheological properties of blood have been justified in different *in vitro* studies. Pentoxifylline decreases blood viscosity, changes the microrheology of erythrocytes by decreasing their aggregation, and improves the deformability of their membranes. These characteristics make pentoxifylline possible to be used as the drug to confirm the validity of the selected test system [21].

Cyproheptadine hydrochloride was investigated in 1 μM. The reference drug pentoxifylline was used in the concentration of 100 μM (the concentration that affects the rheological properties of blood [22]).

For *in vitro* studies, weighed portions of the studied compound or reference drugs were dissolved in distilled water immediately before the experiment. The prepared solutions were instilled into the cuvette by laboratory dispensers for the *in vitro* incubation, and then the studies were carried out according to the design.

Study design

At the first stage, blood samples were collected from the marginal ear veins of the rabbits by the method of free-falling-mass. The blood was stabilized using an aqueous citrate solution (3.8%, pH 6.0) in the ratio of 9:1. To mix the blood with citrate, after filling, the closed tube was immediately gently inverted to the required volume. In this case, the formation of foam should be

prevented from.

At the second stage, the hemorheological activity of RU-31 and the reference drugs were studied, using an experimental model of rabbit blood hyperthermia *in vitro* [23].

For this, the following groups from the obtained samples of blood were formed: an intact control group (6 samples, and each was based on a blood sample of one rabbit); a control group (6 samples: warmed blood samples at 42°C, 1 hour,); there were also three experimental groups: pentoxifylline, RU-31 and cyproheptadine in the studied concentrations were introduced into the blood (6 samples in each group).

The experimental groups, as well as the control group, were subjected to hyperthermia ($t=42^{\circ}\text{C}$, exposure – 1 hour), and the parameters of their blood viscosity, aggregation and deformability of erythrocytes were recorded for each sample.

To study the apparent viscosity, the required volume (2 ml) of blood samples were taken. Then the blood was centrifuged at 3000 rpm for 20 minutes to obtain platelet-poor plasma and erythrocyte mass, which were mixed to obtain blood with a standardized hematocrit of 45%. The hematocrit value was determined as the ratio of the length of the erythrocyte column to the plasma column during centrifugation of capillaries with blood samples (Elmi, Latvia) at 8000 rpm for 3 minutes.

The determination of the apparent viscosity of blood was carried out on a rotary blood analyzer (AKR-2, Russia) at shear rates from 300 s^{-1} to 10 s^{-1} (rotations per second). Based on the obtained data, the erythrocyte aggregation index (EAI, c.u.) was calculated as the ratio of blood viscosity at a shear rate of 10 s^{-1} (the change mainly depends on erythrocyte aggregation) to blood viscosity at 100 s^{-1} (the change of viscosity mainly depends on the deformability of erythrocytes) [24].

The degree of erythrocytes aggregation was determined using the method of optical microscopy (microscope "Biolam Lomo" (Russia). For this, erythrocytes were separated from plasma by centrifugation at 3000 rpm for 20 minutes. Erythrocytes were washed three times in saline and then suspended in autologous plasma. After video recording (digital camera for DCM500 microscope), the number of aggregated and non-aggregated erythrocytes was estimated, and the aggregation index (AI, c.u.) was calculated as the ratio of the number of aggregates to the number of non-aggregated cells [25].

Method for determining the deformability of erythrocytes

The deformability of erythrocytes was assessed by a viscometric method and by visualization in a flow-through microchamber.

The deformability of erythrocytes by a viscometric method, was assessed at a standardized hematocrit of 45%. The viscosity of erythrocyte suspension was mea-

sured on a rotary viscometer at shear rates of 300, 30, 3 s^{-1} .

The elongation of erythrocytes was visualized in the flow-through microchamber which was filled with a suspension of erythrocytes in an isotonic sodium chloride solution containing 0.1% albumin. The pressure applied to the microchamber, thereby created a certain value of the shear stress (τ) in it, which was calculated according to the formula:

$$\tau = \frac{6\eta Q}{Wh^2}$$

where η is the viscosity of the suspension (approximately 1.0 mPa s at 20°C), Q is the volumetric velocity in the microchamber, W is the width of the flow channel of the microchamber, h is the height of the channel equal to the thickness of the gasket.

The image of erythrocytes stretched by a flow of liquid, attached by a single point with the help of human albumin to the bottom of the microchamber, was transmitted from the microscope via a USB port to a computer using a digital eye-piece. After recording the image, it was analyzed in the Adobe Photoshop program (a trial version), where the length and width of the elongated erythrocytes were determined, and the elongation index (EI, c.u.) calculated as an indicator of deformation:

$$EI = \frac{L - W}{L + W}$$

where L is the length of the deformed cell, W is its width [26].

Statistical analysis methods

Statistical data processing was carried out using GraphPad Prism v.8.0 software and Microsoft Office Excel 16. The data are presented as $M \pm m$, where M is the mean values for the group, m is the standard error of the mean. The analysis of the intergroup differences was carried out using nonparametric Mann-Whitney U-test. The differences were determined at the 0.05 significance level.

RESULTS OF THE STUDY

Effect on the viscosity blood characteristics

Heating the control sample of the blood for an hour, led to a significant increase in the blood viscosity (Fig. 1).

Thus, a statistically significant increase in the viscosity of the heated blood samples was revealed in the entire range of the studied shear rates: at the shear rate of 300 s^{-1} , the blood viscosity increased by 10%; at 200 s^{-1} – by 12%, at 100 s^{-1} – by 20%, at 50 s^{-1} – by 19%, at 20 s^{-1} – by 22%, at 10 s^{-1} – by 27%.

When pentoxifylline in the concentration of $100\text{ }\mu\text{M}$, RU-31 and cyproheptadine in the concentration of $1\text{ }\mu\text{M}$ were added to the blood samples and exposed to heat, the general tendency to decrease the blood viscosity was found out (Table 1).

Thus. when pentoxifylline in the concentration of

100 μM was added to the treated blood samples. the general tendency for decrease in blood viscosity at high and low shear rates. was observed. At high shear rates from 300 s^{-1} to 100 s^{-1} . the decrease in blood viscosity in comparison with the control samples was 17% ($p < 0.05$). In a lower range of shear rates. the decrease in blood viscosity was also observed. however. no significant differences in relation to control measurements were found when modeling pathology.

Effected by the action of RU-31 in the concentration of 1 μM . the blood viscosity indices were significantly lower in relation to the control sample at shear rates from 300 s^{-1} to 100 s^{-1} . In average. the decrease was 17% ($p < 0.05$).

In the concentration of 1 μM . cyproheptadine also caused a general tendency for blood viscosity decrease at high shear rates.

Thus. it was revealed that pentoxifylline. the compound of RU-31 and cyproheptadine demonstrate the ability to reduce the viscosity characteristics of blood over the entire range of shear rates. with the most significant differences in the range of shear rates from 300 s^{-1} to 100 s^{-1} . At the same time. in terms of the provided effect. RU-31 is not inferior to pentoxifylline and slightly exceeds the effect of cyproheptadine.

Effect on the parameters of erythrocyte aggregation

Modeling of hyperviscosity led to statistically significant increase in the indices of erythrocyte aggregation in autologous plasma by 54% compared to the intact samples (Fig. 2).

Pentoxifylline. RU-31 and cyproheptadine in the studied concentrations and exposed to heat. added to the blood samples. provide a general tendency to reduce the aggregation of erythrocytes.

Thus. pentoxifylline in the concentration of 100 μM . added to the blood samples subjected to hyperviscosity modeling. statistically significantly decreased in erythrocyte aggregation by 73%.

For the RU-31 compound in the concentration of 1 μM . a statistically significant decrease in this indicator by 70% was registered.

When cyproheptadine was added to the blood samples in the concentration of 1 μM . a significant decrease in erythrocyte aggregation by 65% was observed.

Thus. in the concentration of 1 μM . RU-31 has a pronounced effect on the aggregation ability of erythrocytes in autologous plasma. The level of RU-31 activity is not inferior to pentoxifylline in the concentration of 100 μM and surpasses the reference drug cyproheptadine.

Effect on erythrocyte deformability

The assessment of the studied compounds effect on the deformability of erythrocytes. was carried out by measuring the viscosity of washed erythrocytes suspen-

sion as well as by measuring the degree of erythrocytes deformation in a flow-through microchamber.

When the syndrome of increased viscosity was modeled. a significant increase in the viscosity of a washed erythrocytes suspension was revealed in the entire range of shear rates. So. at the rate of 300 s^{-1} this indicator increased by 10%. at the rate of 30 s^{-1} – by 11%. at the rate of 3 s^{-1} – by 14% ($p < 0.05$) (Table 2).

The addition of pentoxifylline in the concentration of 100 μM to the heated rabbits' blood samples. revealed a significant decrease in viscosity at shear rates of 300 s^{-1} and 3 s^{-1} by 10% and 14%. respectively. The RU-31 compound in the concentration of 1 μM mediated a statistically insignificant decrease in the viscosity parameters of the heated blood samples. For cyproheptadine in the concentration of 1 μM . a statistically significant decrease in viscosity was observed at shear rates of 300 s^{-1} and 3 s^{-1} .

When determining the elongation index of erythrocytes in a flow-through microchamber. it was found out that with the experimental syndrome of the increased blood viscosity. the elongation index of erythrocytes significantly decreased by 32%. The erythrocyte elongation index for pentoxifylline was 0.26 ± 0.010 . i.e. 37% higher than for the control group (modeling of hyperthermia). The compound RU-31 and cyproheptadine did not change the properties of erythrocytes membranes; no statistically significant change for elongation index was found out.

Thus. for RU-31. no statistically significant effects on the viscosity characteristics of the erythrocyte suspension and the structural and functional properties of their membranes was found out.

DISCUSSION

It is known that the rheological status of blood is determined by many factors. including the viscosity of the whole blood. deformability and aggregation of erythrocytes. The thermal action on the blood samples. led to a distinct change in these rheological parameters. As Fig. 1 shows. heating led to a statistically significant increase in the viscosity of the blood samples over the entire range of the studied shear rates. which is presumably associated with a decrease in the deformability of erythrocyte membranes and an increase in their aggregation in the vascular bed [27]. It was found out that the temperature effect on the blood samples contributed to the increase in the aggregation ability of rabbits' erythrocytes by more than twice. as well as to the decrease in the deformability of their membranes by almost 1.5 times. which indicates the decrease in the viscoelastic properties of the erythrocyte membrane.

The revealed changes in the rheological parameters of the blood during modeling the syndrome of increased viscosity. reflect the pathology that develops in the large-diameter vessels and the microvasculature bed [28].

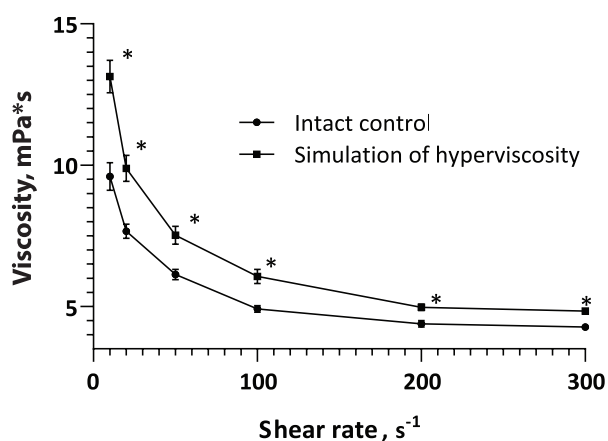


Figure 1 – Simulation of “high viscosity syndrome” (temperature – 42°C, incubation period – 1 hour)

Notes: s⁻¹ – reciprocal seconds. The data are presented as M ± m (mean ± standard error), n = 6. * – the data are reliable in relation to intact blood, with a nonparametric distribution, Mann-Whitney test (p < 0.05)

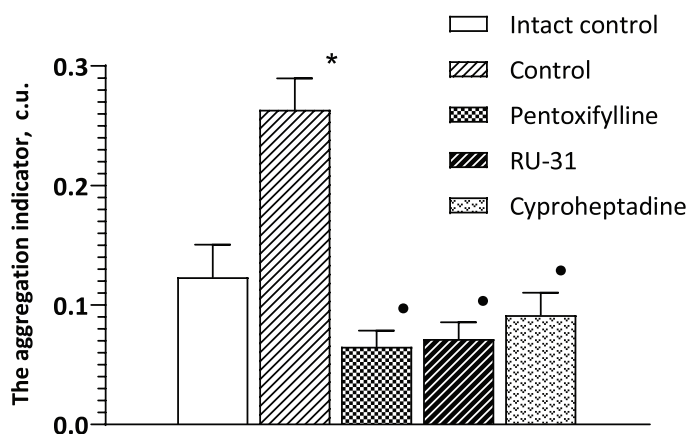


Figure 2 – Effect of pentoxifylline, compound RU-31 and cyproheptadine on aggregation of rabbit erythrocytes after heat exposure

Notes: the aggregation indicator is the ratio of the number of aggregates to the number of erythrocytes. Data are presented as M ± m (mean ± standard error), n = 6. * – the data are reliable in relation to intact blood, with a nonparametric distribution, Mann-Whitney test (p < 0.05). • – differences are significant relative to control measurements (hyperviscosity modeling), with nonparametric distribution, Mann-Whitney U-test (p < 0.05)

Table 1 – Effect of pentoxifylline, RU-31 and cyproheptadine, on the blood viscosity of the rabbits exposed to heat, in order to simulate a “high viscosity syndrome” (temperature – 42°C, incubation period – 1 hour) *in vitro*

Group	mPa*s					
	300 s ⁻¹	200 s ⁻¹	100 s ⁻¹	50 s ⁻¹	20 s ⁻¹	10 s ⁻¹
Control	4.8±0.11	5.0±0.12	6.1±0.26	7.5±0.32	9.9±0.46	13.1±0.58
Pentoxifylline	4.0±0.21*	4.1±0.21*	5.1±0.30*	6.5±0.51	8.6±0.47	11.2±0.87
RU-31	4.1±0.23*	4.2±0.23*	4.9±0.35*	6.3±0.52	8.6±0.75	11.3±1.43
Cyproheptadine	4.2±0.13*	4.3±0.15*	5.2±0.17*	6.8±0.32	9.2±0.41	11.6±0.70

Notes: s⁻¹ – reciprocal seconds. The data are presented as M ± m (mean ± standard error), n = 6. * – the data are reliable in relation to intact blood, with a nonparametric distribution, Mann-Whitney test (p < 0.05)

Table 2 – Influence of RU-31 and reference preparations on the viscosity of washed erythrocytes suspension of rabbits

Group	mPa*s		
	300 s ⁻¹	30 s ⁻¹	3 s ⁻¹
Intact control	2.7±0.06	4.1±0.05	7.2±0.26
Control	3.0±0.02*	4.6±0.15*	8.4±0.18*
Pentoxifylline	2.7±0.10 [•]	4.2±0.24	7.2±0.24 [•]
RU-31	2.9±0.11	4.4±0.29	7.9±0.36
Cyproheptadine	2.8±0.05 [•]	4.5±0.13	7.8±0.11 [•]

Notes: s⁻¹ – reciprocal seconds. The data are presented as M ± m (mean ± standard error). n = 6. * – the data are reliable in relation to intact blood, with a nonparametric distribution. Mann-Whitney U-test (p < 0.05). • – differences are significant relative to control measurements (in hyperviscosity modeling), with nonparametric distribution. Mann-Whitney U-test (p < 0.05)

The experiment revealed that in the concentration of 100 μM, pentoxifylline reduced the blood viscosity in the entire range of the studied shear rates. The most pronounced changes were observed at high shear rates, which is characterized by changes in the components of microrheology.

This was confirmed by the significant decrease in the erythrocyte elongation index, as well as a significant decrease in the rate of their aggregation. The data obtained for the reference drug pentoxifylline, made it possible to confirm the performance of the selected test systems for studying the hemorheological activity of 5-HT₂-antagonists.

During the further work, the data on the effect of RU-31 in the concentration of 1 μM on the whole blood viscosity as well as the erythrocytes aggregation and deformation were obtained. Thus, RU-31 promoted the decrease in blood viscosity over the entire range of the studied shear rates. The most pronounced changes were observed at high shear rates. In addition, the pronounced decrease in the aggregation ability of erythrocytes was notified. The revealed changes in rheological parameters (viscosity of whole blood and erythrocyte aggregation) at high shear rates as well as the decrease in blood viscosity at low shear rates, indicate the ability of RU-31 to improve rheological properties in large-caliber vessels and microvasculature.

In terms of the activity level, RU-31 exceeded the reference drug cyproheptadine and was not significantly inferior to pentoxifylline.

As for the reference drug cyproheptadine in the concentration of 1 μM, it was also characterized by the ability to improve blood viscosity. The most significant changes were recorded at high shear rates. In addition, cyproheptadine slightly improved the aggregation characteristics of erythrocytes.

The ability of type 2A serotonin receptor antagonists to reduce blood viscosity is probably associated with a

possible effect on the plasma component. This was confirmed by the fact that cyproheptadine and RU-31 did not have a pronounced effect on the viscosity of the washed erythrocyte suspension and their deformability, but significantly reduced the aggregation of washed erythrocytes in autologous plasma.

The revealed hemorheological activity of RU-31, comparable to the reference drug pentoxifylline, suggests that this compound is promising for the correction of rheological disorders that provoke a deterioration in cerebral blood flow, which can mediate a decrease in the risk of ischemic stroke in the persons with migraine pathology and stop the aggravation of the pathological processes.

CONCLUSION

Taking into account the data on the participation of 5-HT₂ receptors in the migraine pathogenesis as well as their involvement in the formation of comorbid conditions, it was reasonable to study the effect of type 2 blockers of serotonin receptors, on the viscosity characteristics of blood, aggregation of erythrocytes, as well as their membranes deformability.

The comparative study showed that 5-HT_{2/2A}-antagonists cyproheptadine and RU-31, are able of influencing the rheological properties of the animals' blood with the syndrome of increased blood viscosity *in vitro*.

It was also revealed that the RU-31 compound reduces blood viscosity with the experimental syndrome of increased blood viscosity *in vitro*, and it is practically not inferior to the reference drug pentoxifylline and surpassing the 5-HT₂ blocker cyproheptadine.

Thus, the data obtained in this research, indicate the presence of the ability of 5-HT_{2/2A} antagonists cyproheptadine and RU-31, to influence the rheological properties of blood by reducing viscosity and erythrocytes aggregation, which can expand the range of application of the drugs of this group.

FUNDING

The study was partially carried out within the framework of an internal research grant funded from the funds of Volgograd State Medical University of the Ministry of Health of Russia (Order 29-KO dated 2 Jun 2020).

AUTHORS' CONTRIBUTION

All authors contributed equally to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Harriott AM. Barrett KM Dissecting the association between migraine and stroke. *Current neurology and neuroscience reports*. 2015;15(3):5. DOI: 10.1007/s11910-015-0530-8
2. Gainetdinova DD. Tukhfatullina SI. Clinical and Doppler examination of women with cephalgia and antiphospholipid syndrome. *Journal of Neurology and Psychiatry*. 2019;119(7):48–53. Russian
3. Tabeeva GR. Migraine and stroke. *Consilium Medicum*. 2010;12(2):17–22. Russian
4. Raut S. Singh U. Sarmah D. Datta A. Baidya F. Shah B. Bohra M. Jagtap P. Sarkar A. Kalia K. Borah A. Dave KR. Yavagal DR. Bhattacharya P. Migraine and Ischemic Stroke: Deciphering the Bidirectional Pathway. *ACS Chem Neurosci*. 2020;11:1525–1538. DOI: 10.1021 / achemneuro.0c00137
5. Sadokha KA. Evstigneev VV. Golovko AM. Kisten OV. Migraine stroke: myth or reality. *Medical News*. 2019;1:12–18. Russian
6. Tabeeva GR. Cerebrovascular disorders associated with migraine. *Medical Council*. 2017;10:328–330. DOI: 10.21518 / 2079-701X2017-10-32-35. Russian
7. Mawet J. Kurth T. Ayata C. Migraine and stroke: in search of shared mechanisms. *Cephalalgia*. 2015;35(2):165–81. DOI: 10.1177/0333102414550106.
8. Lowe GDO. *Clinical blood rheology*. Crc Press. 2019;2:245.
9. Forman SB. Roy K. Vasoactive and antiplatelet agents. *Comprehensive Dermatologic Drug Therapy E-Book*. 2019;358.
10. Spasov AA. Yakovlev D. Brigadirova A. Maltsev D. Agatsarskaya Y. Novel Approaches to the Development of Antimigraine Drugs: A Focus on 5-HT_{2A} Receptor Antagonists. *Russian Journal of Bioorganic Chemistry*. 2019;45:76–88. DOI: 10.1134/S1068162019020146. Russian
11. Spasov AA. Yakovlev DS. Maltsev DV. Miroshnikov MV. Sultanova KT. Zhukovskaya ON. Anisimova VA. Nechaeva KA. The neurotoxicological profile of the 5-HT_{2A} antagonist of the imidazobenzimidazole derivative. *Toxicological Bulletin*. 2019;154(1):23–28. DOI: 10.36946/0869-7922-2019-1-23-28. Russian
12. Sultanova KT. Yakovlev DS. Maltsev DV. Miroshnikov MV. Morkovina YaV. Anisimova VA. Morkovnik AS. Anxiolytic properties of compound RU-31. *Journal of VolgSMU*. 2018;67(3):28–32. DOI: 10.19163/1994-9480-2018-3(67)-28-32. Russian
13. Yakovlev DS. Spasov AA. Mal'tsev DV. Anisimova VA. Effect of 5-HT (2A) receptor antagonists on blood flow in the carotid vessels upon elevation of serotonin level. *Bull Exp Biol Med*. 2014;157(3):350–2. DOI: 10.1007/s10517-014-2563-4. Russian
14. Mkhitarian EA. Naftidrofuril in the treatment of cerebrovascular diseases. *Neurological journal*. 2015;20(6):41–44. Russian
15. Noble MIM. Drake-Holland AJ Preservation of haemostasis with anti-thrombotic serotonin antagonist. *J Hematol Clin Res*. 2017;1:019–025. DOI: 10.29328/journal.jhcr.1001004
16. Ogawa T. Sugidachi A. Tanaka N. Fujimoto K. Asai F. Effects of R-102444, an orally active 5-HT_{2A} receptor antagonist, in rat models of peripheral vascular disease. *Vascul Pharmacol*. 2004;41(1):7–13. DOI: 10.1016/j.vph.2004.03.001
17. Agatsarskaya YV. Yakovlev DS. Maltsev DV. Semenova YuV. Salikhov DA. Sultanova KT. Anisimova VA. Neuroreceptor effects of antimigraine agent 9-diethylaminoethyl-2-(4-methoxyphenyl)imidaz[1,2-a]benzimidazole. *Journal of VolgSMU*. 2019;69(1):120–124. DOI: 10.19163/1994-9480-2019-1(69)-120-124. Russian
18. Spasov AA. Anisimova VA. Yakovlev DS. Petrov VI. Minkin VI. Medications exhibiting properties of antagonists of serotonin 5-HT_{2A}- receptors. Patent of Russia No.2465901. 2012. Russian
19. Spasov AA. Yakovlev DS. Maltsev DV. Zhukovskaya ON. Anisimova VA. Kovalev GI. Zimin IA. Morkovina YV. The derivatives of imidazo[1,2-a] benzimidazole as 5-HT_{2A} receptor antagonists. *Russian Journal of Bioorganic Chemistry*. 2016;42(4):397-403. DOI: 10.1134 / S1068162016040178. Russian
20. Spasov AA. Naumenko LV. Khaliullin FA. Study of the mechanism of hemobiological action of a new xanthine derivative. *Volgograd Journal of Medical Scientific Research*. 2006; 4:3–5. Russian
21. Plotnikov MB. Aliev OI. Plotnikova TM. Methodological approaches to the study of substances affecting blood rheology. *Experimental and Clinical Pharmacology*. 2011;74(12):36–39. Russian
22. Muravyov AV. Roitman EV. Levin VN. Maimistova AA. Bulaeva SV. Chuchkanov FA. Zamyshlyayev AV. Changes in the rheological properties of blood in persons with cerebral atherosclerosis in conditions of drug therapy with pentoxifylline (trental). *Thrombosis. hemostasis and rheology*. 2010;2:28–33. Russian
23. Dintenfass L. Modifications of blood rheology during aging and age-related pathological conditions. *Aging (Milano)*. 1989;1(2):99–125. DOI: 10.1007/BF03323882
24. Lopukhin YuM. Parfenov AS. Peshkov FI. AKR-2 blood viscosity analyzer. Determination of rheological properties of blood. Methodical recommendations. Scientific Research Institute of Phys.-Chem. medicine. 1996;15. Russian
25. Dvoryansky SA. Tsirkin VI. Methods for studying the aggregation ability of erythrocytes. *Vyatka medical bulletin*. 2008;3–4.
26. Muravyov A et al. Methods for studying the deformability of erythrocytes in experiment and clinic. *Clinical laboratory diagnostics*. 2010;1:28–32.
27. Podzolkov VI. Koroleva TN. Pisarev MV. Kudryavtseva MG. Zateishchikova DA. Disturbances of microcirculation and

- functional state of erythrocytes as a factor of cardiovascular risk in metabolic syndrome. Rational Pharmacotherapy in Cardiology. 2018;14(4):591–597. DOI: 20996 / 1819-6446-2018-14-4-591-597
28. Popelnyuk NS. Davydkin IL. Kozlova OS. Krivova SP. Kuzmina TP. Naumova KV. The problem of studying the processes of microcirculation and blood rheology in the clinic of internal diseases. Ural Medical Journal. 2019;8:25–29. DOI: 10.25694 / URMJ.2019.08.22
29. Muravyov AV. Mikhailov PV. Tikhomirova IA. Microcirculation and hemorheology: points of interaction. Regional blood circulation and microcirculation. 2017;16(2):90–100.

AUTHORS

Dmitry S. Yakovlev – Doctor of Sciences (Medicine). Associate Professor. Professor of the Department of Pharmacology and Bioinformatics of Volgograd State Medical University. ORCID ID: 0000-0001-8980-6016. E-mail: dypharm@list.ru

Lyudmila V. Naumenko – Doctor of Sciences (Medicine). Associate Professor. Professor of the Department of Pharmacology and Bioinformatics of Volgograd State Medical University. E-mail: milanaumenko@mail.ru

Kira T. Sultanova – Assistant of the Department of Pharmacology and Bioinformatics of Volgograd State Medical University. ORCID ID: 0000-0002-9846-8335. E-mail: sultanova.pharma@gmail.com

Alexander A. Spasov – Doctor of Sciences (Medicine). Academician of the Russian Academy of Sciences. Professor. the Head of the Department of Pharmacology and Bioinformatics of Volgograd State Medical University. ORCID ID: 0000-0002-7185-4826. E-mail: aspasov@mail.ru