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Research Article

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Endothelial dysfunction: comparative evaluation of ultrasound dopplerography, laser dopplerflowmetry and direct monitoring of arterial pressure for conducting pharmacological tests in rats

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Abstract

Introduction: The importance of the problem of endothelial dysfunction makes it essential to improve approaches to its evaluation. Due to the high diagnostic potential of ultrasound dopplerography, this method is promising in the study of endothelial function.

Objective:To study endothelial function when conducting vascular pharmacological tests in the norm and with the blockade of the nitric oxide synthesis by means of the ultrasound method for examining the central blood flow velocity in the femoral artery of the rat in comparison with changes of systemic haemodynamics and microcirculation velocity in the medial vastus muscle using laser Doppler flowmetry.

Materials and methods: In the norm and in conditions of deficiency of endogenous nitric oxide, three methods for assessing haemodynamics were used simultaneously: 1) dopplerography using the ultrasoundDoppler system (Minimax-Doppler-K, St Petersburg); 2) laser doppler flowmetry using the TDS144 sensor (Biopac MP-150, USA); 3) recording the arterial pressure in the carotid artery by means of a catheter connected to a TDS-160-A sensor (Biopac MP-150, USA). To study the vasoregulatory function of the endothelium under conditions of multicomponent monitoring of haemodynamics, functional vascular tests were performed using acetylcholine and sodium nitroprusside.

Results: Quantification of endothelial dysfunction when conducting pharmacological tests with endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilation is most significant when calculating the coefficient reflecting the ratio of areas of vascular response. Theblood flow velocity parameters recorded by using Doppler ultrasound showed a high correlation level with both arterial pressure and with a linear microcirculation rate. The most comprehensive dopplerographic indices for evaluating the function of the endothelium by conducting pharmacological tests in the norm and in the case of pathology, are the estimated systolic-diastolic difference in blood flow velocities (ΔVs-Vd) in the femoral artery. The study of the endothelial function when conducting vascular pharmacological tests in the norm and with nitric oxide synthesis blockaded, by means of the ultrasound examination of the central blood flow velocity in the femoral artery, is comparable to changing the haemodynamics parameters as well as velocity in microcirculation of the medial vastus muscle using laser Doppler flowmetry.

Conclusions: Ultrasonic dopplerography using the Minimax-Doppler-K device reflects the systemic and local vascular response to the administration of vasodilators both in the norm and with the blockade of the nitric oxide synthesis and makes it possible to conduct an informative assessment of the endothelial function.

Keywords

Minimax-Doppler, microcirculation, endothelial dysfunction, rats, nitric oxide, acetylcholine, L-NAME.

Introduction

Antiarrhythmic drugs have been in use for over 100 years to prevent and treat heart rhythm disorder despite many limitations and adverse effects (Castro et al. 2015). Their role of being the essential part of antiarrhythmic therapy inspires researchers to create novel molecules all over the world.

The mechanism of action is the cornerstone, which determines the therapeutic effectiveness and safety profile of an antiarrhythmic drug. It might be defined as an assembly of functional transformations of intracellular homeostasis and extracellular interaction that ultimately determines the pharmacological Ultrasonic dopplerography is a valuable diagnostic tool that allows the assessment of the functional state of the vasculature in the norm and in the case of pathology (Petrishchev and Vasina 2009, Tyurenkov and Voronkov 2006). The main advantage of the ultrasound examination is the possibility of non-invasive assessment of blood flow velocity in large vessels. The dynamics of the blood velocity in the vessels is affected by a large number of factors: from the laws of hydrodynamics to subtle molecular mechanisms for regulating systemic and regional blood flows. It is known that dopplerography does not reflect the actual blood flow velocity, as the values obtained depend on the insonisation angle. Due to this and when using this method, preference is given to the analysis of relative indices, which can be obtained when conducting various tests, mostly pharmacological tests.

In addition, in clinical settings where sampling is not always possible, the use of the Pourcelot index (RI, resistivity index) and the Gosling index (PI, pulsatility index) has become widespread; they allow not only for the position of the blood flow velocity curve relative to zero, but also for the proportions of its individual parts. The value of the Pourcelot index is determined by the ratio (Vs-Vd)/Vs, whereas the Gosling index is estimated as (Vs-Vd)/Vmean, where Vs is the systolic blood flow velocity, Vd is the diastolic blood flow velocity and Vmean is the area of one peak of the velocity curve. In most cases, these indices adequately reflect the patency of the vascular network distal to the point of registration. However, some authors emphasise that their sensitivity varies depending on the study design and is applicable only to a limited range of models (Adamson and Langille 1992).

The purpose of this study is to optimise the approach for assessing the endothelial dysfunction in the case of ultrasound recording of blood flow velocity by studying the correlation between the dynamics of blood flow velocity changes in the femoral artery, arterial pressure and linear microcirculation rate when undertaking functional tests in the presence of activation and blockade of endogenous nitric oxide synthesis.

Materials and Methods

The study was conducted on 18 male Wistar rats weighing 180-210 g, which were all obtained from The Stolbova nursery of the RussianAcademy of Medical Sciences (Moscow region) at the same time. All activities with the animals were carried out in compliance with *The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (Directive2014/63/EU). All the experiments were approved by the local Ethics Committee (Minutes No. 7-2017 of 11 September 2017).

The animals were divided into two equal groups: 1) intact; and 2) with nitro-L-arginine-induced (L-NAME-induced) endothelial dysfunction (by administering L-NAME intraperitoneally 25 mg/kg once a day for seven days) (Pokrovskij et al. 2006).

After the anaesthetised rats (chloral hydrate 150 mg kg, zoletil 60 mg/kg) had been fixed on the stage, their right carotid artery and their right femoral neurovascular bundle were isolated and their left femoral vein was catheterised.

For a multicomponent study of haemodynamic parameters affected by the administration of acetylcholine and sodium nitroprusside, the systemic arterial pressure, the velocity of blood flow in the femoral artery and the rate of microcirculation in the thigh muscle tissue, which the femoral artery supplies with blood, were recorded. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a TDS-160-A sensor (Biopac, software – AcqKnowledge 4.2) connected to a catheter in the right carotid artery. The velocity of blood in the femoral artery was measured by means of a UZOP-010-01 sensor with a working frequency of 25 MHz of a Minimax-Doppler-K computer-aided dopplerograph with software MM-D-K-Minimax Doppler v.2.1 (St. Petersburg, Russia). After setting the sensor above the middle third of the right femoral artery, the following parameters were recorded: maximum systolic (Vs) and diastolic velocity (Vd), maximum systolic and diastolic velocities on the curve of average blood flow velocity (Vas, Vad), mean velocity on the curve of average blood flow velocity (Vam), mean and bulk velocity on the average velocity curve (Qas, Qam), pulsatility index (PI, Gosling index) and resistivity index (RI, Pourcelot index). To measure the speed of the formed elements in the microcirculatory bloodstream (a linear microcirculation rate), a needle probe TDS144 (Biopac, USA) was placed in the lower third of the medial vastus muscle. The vasoactive agents (acetylcholine – 40 μg/kg, sodium nitroprusside – 30 μg/kg) were injected through a catheter placed in the left femoral vein consecutively with

an interval of 15 minutes (Galagan et al. 1991, Liauder et al. 2000).

Thus, the possibility of concurrent monitoring of the parameters for haemodynamics and microcirculation at three levels in the system "centre – elastic vessel – peripheral blood flow" was obtained. Statistical processing of the results was carried out by means of STATISTICA 10.0 and Microsoft Excel 2013. A statistical analysis for the correlation was made using Spearman's rank test. The significance of the intergroup differences was statistically tested by the Student's test.

Results

The intact rats gave the same response to the administration of acetylcholine and sodium nitroprusside, which showed in: 1) a decrease in of SBP, DBP, mean BP, linear microcirculation rate, diastolic blood flow velocity in the femoral artery (Vd, Vad); 2) an increase in systolic blood flow velocity in the femoral artery (Vs, Vas), Pourcelot index and Gosling index (Fig. 1). Most of the parameters returned to their original values within 2 minutes.

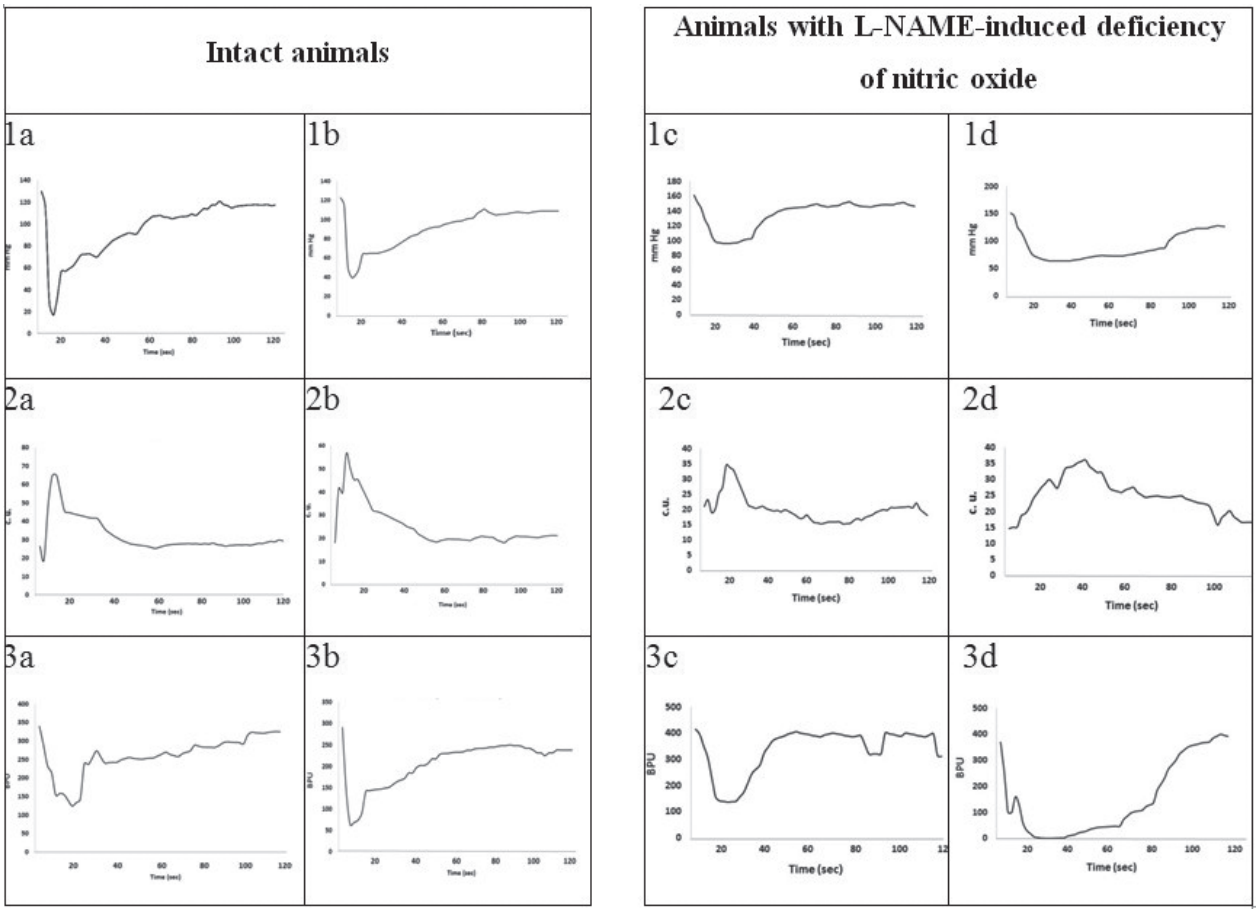

Figure 1. Endothelium-dependent (acetylcholine) and endothelium-independent (nitroprusside) vascular reactions in intact rats (**a, b)** and affected by administration of L-NAME (**c, d**). (**1a**) dynamics of the mean BP when administering acetylcholine to the intact animals; (**2a**) dynamics of the maximum systolic velocity recorded above the femoral artery when administering acetylcholine to the intact animals; (**3a**) dynamics of the linear microcirculation rate in the lower third of the medial vastus muscle when administering acetylcholine to the intact animals; (**1b**) dynamics of the mean BP when administering sodium nitroprusside to the intact animals; (**2b**) dynamics of the maximum systolic velocity recorded above the femoral artery when administering sodium nitroprusside to the intact animals; (**3b**) dynamics of the linear microcirculation rate in the lower third of the medial vastus muscle when administering sodium nitroprusside to the intact animals; (**1c**) dynamics of the mean BP when administering acetylcholine to the animals with L-NAME-induced deficiency of nitric oxide; (**2c**) dynamics of the maximum systolic velocity recorded over the femoral artery when administering acetylcholine to the animals with L-NAME-induced deficiency of nitric oxide; (**3c**) dynamics of the linear microcirculation rate in the lower third of the medial vastus muscle when administering acetylcholine to the animals with L-NAME-induced deficiency of nitric oxide; (**1d**) dynamics of mean BP when administering sodium nitroprusside to the animals with L-NAME-induced deficiency of nitric oxide; (**2d**) dynamics of the maximum systolic velocity recorded over the femoral artery when administering sodium nitroprusside to the animals with L-NAME-induced deficiency of nitric oxide; (**3d)** dynamics of the linear microcirculation rate in the lower third of the medial vastus muscle when administering sodium nitroprusside to the animals with L-NAME-induced deficiency of nitric oxide.

For further statistical processing, a number of values over time for each sample were expressed as a percentage, where the initial value was taken as 100% according to the formula: value/initial value×100%. Statistical analysis using the Spearman's rank test showed that, in both samples, the dynamics of many of the parameters under study correlated reliably between each other (Table 1). Taking into consideration that, when conducting the functional tests,an increase in systolic and a decrease in diastolic blood flow were recorded, the systolic-diastolic blood flow velocity difference (ΔVs-Vd) was included in the statistical analysis as a parameter to integrate parameters Vs and Vd, as it is potentially more sensitive than the latter parameters.

The highest degree of correlation at $p<0.05$ was found between such indicators as mean BP, linear microcirculation rate, systolic velocity and systolic-diastolic velocity difference in the femoral artery (Vs, ΔVs-Vd) (Figs. 2, 3 and 4) (Table 1). The other dopplerographic parameters (Vas, Vad, Vm, Vam, Qs, Qas, Qam) demonstrated a low and less reliable correlation with the dynamics of arterial blood pressure and microcirculation rate.

As in the group of the intact animals, in the group of the animals with L-NAME-induced endothelial dysfunction, the greatest correlation with the indicators of systemic and local haemodynamics was found for such dopplerographic parameters as Vs, Vd and ΔVs-Vd.

The obtained results indicate that the blood flow parameters Vs and ΔVs-Vd, recorded by means of a Minimax-Doppler-K device, reliably correlate with the data recorded when studying both systemic haemodynamics and the reaction of the microcirculatory bloodstream to the administration of acetylcholine and sodium nitroprusside.

Using regression analysis, the hypothesis that the relationship between the dynamics of the parameters under study obeys a linear equation was tested. Using STATISTICA 10.0 software, the formulae describing the dependence of the parameters under study for the following pairs are defined: 1) Vs – Mean BP; 2) Vs – Linear microcirculation rate; 3) ΔVs-Vd – Mean BP; 4) ΔVs-Vd – Linear microcirculation rate. In all cases, the variables obeyed the following Formula (1):

$$y = k_1 x + k_2 x^2 + m \quad (1)$$

where, x –Vs, ΔVs-Vd;
Y – mean BP, Linear microcirculation rate;
k_1 k_2, m – empirically calculated coefficients

The values of k_2 were significantly smaller than those of k_1 (5-6 orders of magnitude less), which indicates their insignificant contribution and makes it possible to neglect them and to form an opinion about the predominance of the linear form of the dependence between the parameters under study. This circumstance makes it clear that their changes occur proportionally regardless of the initial values.

Since the endothelial function can be assessed by the difference in responses to the endothelium-dependent and endothelium-independent test, the next step was a quantitative analysis of the results obtained and an assessment

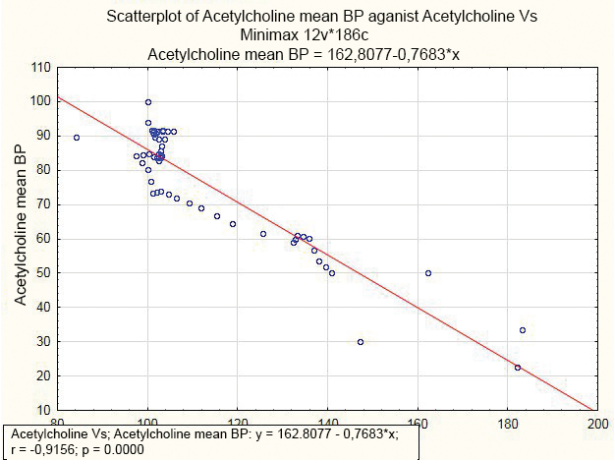

Figure 3. Scatter graph, representing the dependence of the mean BP and the maximum systolic velocity when administering acetylcholine to the intact animals.

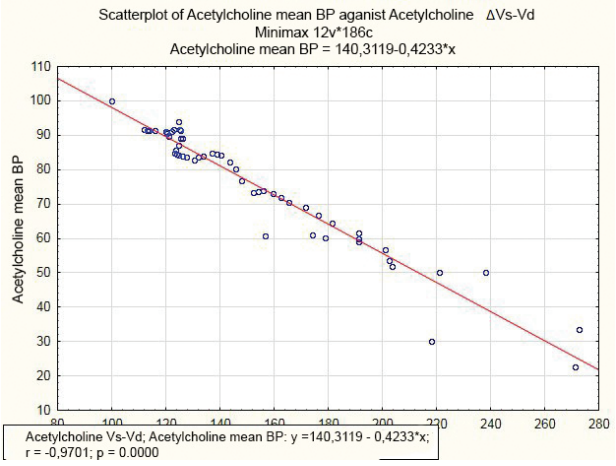

Figure 2. Scatter graph, representing the dependence of the mean BP and systolic-diastolic velocity difference when administering acetylcholine to the intact animals.

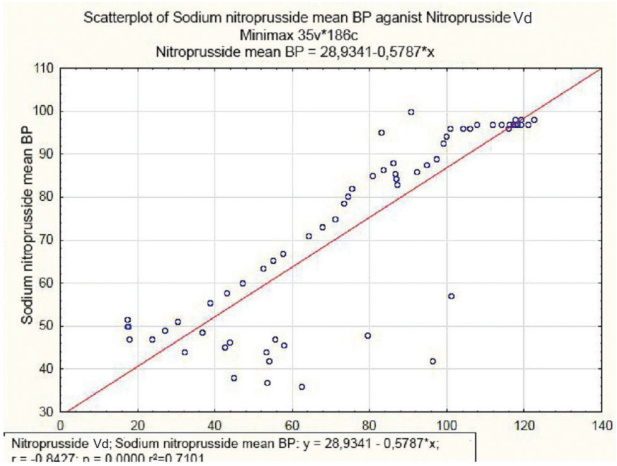

Figure 4. Scatter graph, representing the dependence of the mean BP and maximum diastolic velocity when administering sodium nitroprusside to the intact animals.

of the intensity of the reactions recorded when measuring the central and peripheral parameters of haemodynamics. Using Microsoft Excel 2013 software, the areas formed by the curves of changes in arterial blood pressure, microcirculation rate, Vs and ΔVs-Vd after administering the pharmacological agents, were calculated. The area was determined by applying the standard formula as the sum of the areas of rectangular trapezoids, forming a figure bounded by a curve (Formula 2 and Formula 3).

$$S = \sum_{n=1}^{120} [(X_{n-1} - 100) + (X_n - 100)] * t_n/2 \quad (2)$$

$$S = \sum_{n=1}^{120} [(100 - Y_{n-1}) + (100 - Y_n)] * t_n/2 \quad (3)$$

where X – Vs, ΔVs–Vd at a time point n, where n = {0, 2, 4,…,120}, t = 2 seconds and X_0 was taken as 100% and Y – SBP, DBP, Linear microcirculation rate at a time point n, where n = {0, 2, 4, ..., 120}, t = 2 seconds and Y_0 was taken as 100% (Fig. 5).

Then, for the parameters under study, the average ratio S_{NP}/SACwas determined, where in the Doppler study, S_{NP} – the area under the rise curve of the values of Vs and ΔVs-Vd over time when administering nitroprusside; when studying the parameters of central haemodynamics – the area above the decline curve of mean BP, when employing laser Doppler flowmetry – the area above the decline curve of the linear microcirculation rate. S_{AC} is the area under the rise curve of the values of Vs and ΔVs-Vd over time when administering acetylcholine; when studying the parameters of central haemodynamics – the area above the decline curve of the mean BP, when employing laser Doppler flowmetry –the area above the decline curve of the linear microcirculation rate. The time during which this calculation of areas was carried out was limited to 120 seconds after the administration of sodium nitroprusside and acetylcholine (Table 2).

A statistical analysis of significance of differences in $S_{NP}/S_{AC,}$ calculated by the parameters Vs, ΔVs-Vd, mean BP and linear microcirculation rate using the Student's test (with Bonferroni's correction),was carried out. When carrying out an intergroup pairwise comparison between the intact group and the group with L-NAME-induced deficiency of nitric oxide, it was identified that the ratios of S_{NP}/S_{AC} calculated for each parameter are significantly different ($p<0.05$) (Figure 6).

Discussion

Earlier in our laboratory, it was shown that, when undertaking functional vascular tests with a prolonged, within 7 days, intraperitoneal administration of L-NAME at a dose of 25 mg/kg in laboratory animals, there appeared a complex of functional and morphometric changes associated with the development of a deficiency of nitric oxide due to the blockade of NO-synthase. The dynamics of arterial pressure indices was assessed by calculating the areas of the triangles above the reaction curve for restoring the mean arterial pressure after the administration of nitroprusside and above the reaction curve for restoring the mean arterial pressure after the administration of acetylcholine. To evaluate the endothelial function, the endothelial dysfunction coefficient was proposed, which is determined by calculating the ratio between the area of the triangle above the reaction curve for restoring the mean arterial pressure after the administration of nitroprusside

Table 1. Spearman's Correlation Coefficients Revealing the Link Between the Dynamics of Changing Values Obtained When Measuring mean BP, Linear Microcirculation Rate and Doppler Sonography (Vs, Vd and ΔVs-Vd, PI and RI) When Vascular Sampling in the Group of Intact Rats (n = 9) and in Group of Rats with L-NAME-induced Deficiency of Nitric Oxide (n = 9).

Correlation coefficients between the studied parameters when administering acetylcholine to intact animals						
Compared parameters	**Vs**	**Vd**	**ΔVs–Vd**	**RI**	**PI**	**Mean BP**
Mean BP	-0.72*	0.69*	-0.86*	-0.71*	-0.71*	1
Linear microcirculation rate	-0.46*	0.39*	-0.52*	-0.45*	-0.49*	0.57*
Correlation coefficients between the studied parameters when administering sodium nitroprusside to intact animals						
Compared parameters	**Vs**	**Vd**	**ΔVs–Vd**	**RI**	**PI**	**Mean BP**
Mean BP	-0.71*	0.69*	-0.82*	-0.69*	-0.68*	1
Linear microcirculation rate	-0.49*	0.41*	-0.51*	-0.68*	-0.61*	0.69*
Correlation coefficients between the studied parameters when administering acetylcholine to animals with L-NAME-induced Deficiency of Nitric Oxide						
Compared parameters	**Vs**	**Vd**	**ΔVs–Vd**	**RI**	**PI**	**Mean BP**
Mean BP	-0.71*	0.68*	-0.85*	-0.76*	-0.71*	1
Linear microcirculation rate	-0.49*	0.41*	-0.52*	-0.42*	-0.40*	0.59*
Correlation coefficients between the studied parameters when administering sodium nitroprusside to animals with L-NAME-induced Deficiency of Nitric Oxide						
Compared parameters	**Vs**	**Vd**	**ΔVs–Vd**	**RI**	**PI**	**Mean BP**
Mean BP	-0.72*	0.71*	-0.86*	-0.70*	-0.73*	1
Linear microcirculation rate	-0.51*	0.41*	-0.52*	-0.41*	-0.41*	0.54*

Key: Vs – maximum systolic velocity; Vd – maximum diastolic velocity; ΔVs-Vd – systolic-diastolic velocity difference; PI – pulsatility index; RI –resistivity index; MAP – mean BP. The "-" sign means that, with an increase in one indicator, there is a decrease in the compared indicator (negative correlation). * – $p<0.05$.

and the area of the triangle above the reaction curve for restoring the mean arterial pressure after the administration of acetylcholine (Kochkarov et al. 2006, Pokrovskaya et al. 2007, Pokrovskij et al. 2010, Pokrovskii etal. 2009). As a result, it amounted to 1.1± 0.1 in the intact rats and 5.4±0.6 in the group of animals with L-NAME-induced deficiency of nitric oxide.

In this study, in order to analyse the data obtained, the area of reactions under the curve of Vs and ΔVs-Vd, over the curve of mean BP, the linear microcirculation rate after the administration of sodium nitroprusside and acetylcholine and their ratio in the case of endothelium-dependent and endothelium-independent vasodilation in the intact rats and in the group of animals with L-NAME-induced deficiency of nitric oxide, was calculated. A significant difference was found between the systolic blood flow velocity and systolic-diastolic difference in femoral blood flow velocity, the linear microcirculation rate, as well as above the curve of dynamics of the mean BP between the group of the intact animals and the group of animals with L-NAME-induced endothelial dysfunction. Simulating endothelial dysfunction led to a significant increase in the ratio of reaction areas, which amounted to 3.94±0.27 for Vs, 3.99±0.29 for ΔVs-Vd, 4.05±0.31 for mean BP; 4.11±0.52 for the linear microcirculation rate and these values confirm the equivalence of the research methods chosen for assessing the endothelial function.

In the multicomponent study of haemodynamic parameters and microcirculation affected by undertaking pharmacological tests, the greatest correlation between the blood flow velocity parameters of the femoral artery (Vs and ΔVs-Vd), the dynamics of the mean arterial blood pressure and the linear microcirculation rate, was observed both in the group of the intact animals and in the animals with L-NAME-induced deficiency of nitric oxide. The parameter ΔVs-Vd showed the greatest degree of correlation with the mean arterial blood pressure, which can be due to the fact that the shape of the Vs curve

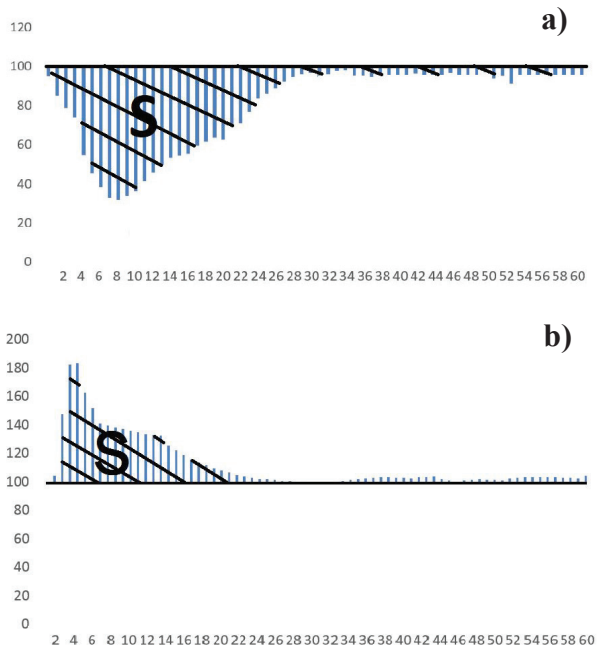

Figure 5.**a**) Example of calculating the area above the decline curve of the studied parameter; **b**) Example of calculating the area underthe rise curve of the studied parameter.

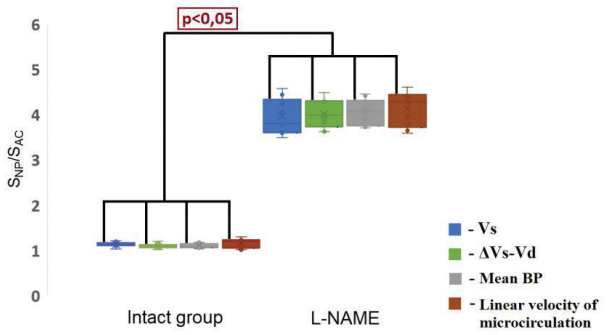

Figure 6. Intergroup pairwise comparison of the group of intact animals with the group of animals with L-NAME-induced deficiency of nitric oxide by the following parameters under study: Vs, ΔVs-Vd, mean BP and linear microcirculation rate.

Table 2. Reaction Areas for the Parameters Under Study and Their Ratio When Administering Acetylcholine and Sodium Nitroprusside to the Group of Intact Rats (n = 9) and to the Group of Animals with L-NAME-induced deficiency of nitric oxide (n = 9).

Reaction Areas for the Parameters Under Study and Their Ratio When Administering Acetylcholine and Sodium Nitroprusside to the Group of Intact Rats				
Reaction area	**Vs**	**ΔVs–Vd**	**Mean BP**	**Linear microcirculation rate**
S_{AC}	1801.3±92.25	1987.2±123.13	1254±101.84	2357.9±201.13
S_{NP}	1910.5±121.14	2165.4±121.80	1369.1±122.70	2601.6±256.53
S_{NP}/S_{AC}	1.12±0.06	1.10±0.06	1.09±0.05	1.15±0.11
Reaction Areas for the Parameters Under Study and Their Ratio When Administering Acetylcholine and Sodium Nitroprusside to the Group of Animals with L-NAME-induced deficiency of nitric oxide				
Reaction area	**Vs**	**ΔVs–Vd**	**Mean BP**	**Linear microcirculation rate и**
S_{AC}	842.3±32.23	940.2±43.12	709±93.90	1007.8±199.32
S_{NP}	2501.5±132.43	2798.4±143.76	1969.1±223.62	3211.5±356.57
S_{NP}/S_{AC}	3.94±0.27	3.99±0.29	4.05±0.31	4.11±0.52

Key: henceforward, S_{NP} – the area under the curve of Vs and ΔVs-Vd and above the curve of mean BP, linear microcirculation rate after administration of sodium nitroprusside; S_{AC} – the area under the curve of Vs and ΔVs-Vd and above the curve of mean BP, linear microcirculation rate calculated after the administration of acetylcholine.

depends more on the level of the initial ABP, whereas Vd is more susceptible to external interference (breathing excursions, vibrations etc.) because its absolute values are an order of magnitude less than the values of other parameters.

Despite the fact that the resistivity and pulsatility index consider the difference component of Vs and Vd, the dynamics of their change was less informative. A decrease in the indices is believed to correlate with a drop in vascular resistance, but in this study, when performing pharmacological tests in both groups, there was an increase in these indicators. The data obtained can be explained by a decrease in Vd. As a consequence, both the denominator and, to a greater extent, the numerator of the resistivity and pulsatility indices increase, these being determined respectively by the relations (Vs-Vd)/Vmean and (Vs-Vd)/Vs. The results obtained are consistent with the data in Adamson and Langille 1992 and can be explained by the polar difference in the variation of these indices with local and generalised vasodilation. After systemic administration of vasodilators, the volume of circulating blood is redistributed towards the microcirculatory bloodstream. These processes lead to a decrease in diastolic velocity and an increase in the amplitude of the peaks of the blood flow velocity curve, which causes an increase in both indices, despite a reduced vascular resistance (Adamson and Langille 1992).

The combined evaluation of the results obtained suggests that non-invasive ultrasound dopplerography using the ΔVs-Vd index integrates the dynamics of arterial blood pressure and microcirculation in it, which is confirmed by carrying out a correlation analysis and by calculating the reaction areas of the studied blood flow velocity indicators, which in turn confirms the possibility of applying this index for assessing the endothelial function.

Conclusions

1. When calculating the ratio of areas of S_{NP}/S_{AC} under the curve of the dynamics of systolic blood flow velocity changes and systolic-diastolic difference in blood flow velocities in the femoral artery, the linear microcirculation rate, as well as that above the curve of the dynamics of the mean BP change between the group of the intact animals and the group of the animals with L-NAME-induced endothelial dysfunction, a significant difference in the studied parameters was found. The above-mentioned confirms the possibility of applying a calculated ratio of reaction areas under the curve of systolic-diastolic difference in blood flow velocities after the administration of sodium nitroprusside and acetylcholine to assess the endothelial function.
2. In conducting functional vascular tests, both in the group of the intact animals and in the animals with L-NAME-induced deficiency of nitric oxide, a high degree of correlation was found between the changes in mean BP, systolic blood flow velocity (Vs) and systolic-diastolic blood flow velocity difference (ΔVs-Vd).

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