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Ultrasonic dopplerography for the evaluation of endothelial function in the conduct of pharmacological vascular samples in an experiment

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ABSTRACT

To study the function of the endothelium in conducting vascular pharmacological samples in normal and blocking the synthesis of nitric oxide by ultrasound examination of the velocity of central blood flow in the femoral artery in comparison with the changes in systemic hemodynamics and the linear velocity of microcirculatory flow using laser flowmetry. It was studied the parameters of the blood flow velocity (the maximum systolic and diastolic velocity, average systolic and diastolic velocity, Gosling index of pulsatility and resistance index by the Doppler ultrasound Minimax-Doppler-K), the parameters of central circulation ("Biopac-systems MP-150", AcqKnowledge 4.2, USA), and linear velocity of the microcirculatory flow of the musculus vastus medialis (Biopac MP-150, USA) in intact animals and rats with N-Nitro-L-arginine methyl ester (L-NAME) induced deficiency of nitric oxide. Quantitative evaluation of endothelial dysfunction in the conduct of pharmacological tests with endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilatation is most indicative in calculating the coefficient reflecting the ratio of vascular responses areas in intact rats with L-NAME induced deficiency of nitric oxide. Blood flow parameters (the maximum systolic velocity, the calculated difference between systolic and diastolic velocities) showed a high correlation, both with mean arterial pressure and a linear velocity of the microcirculatory flow. We conclude, that ultrasound Doppler were reflected in the systemic and local vascular response to the administration of vasodilators. It allows an assessment of endothelial function by using the Minimax-Doppler-K device.

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INTRODUCTION

The key of the cardiovascular diseases development is atherosclerotic vascular lesions. There is a disruption of endothelial function in the early stages (*Soboleva, G.N et al., 2010)*. The search for both innovative molecules (*Kravchenko, D.V., et al., 2016; Bogus, S.K., et al., 2017*) and methods for identifying endothelial dysfunction is an important goal of modern medicine. In this case, the study should be conducted on pharmacological targets (Ragulina, V.A *et al.,* 2017; Danilenko, L.M *et al.,* 2016), *in vivo* models (*Molchanova, O.V et al., 2016; Shakhno, E.A. et al., 2016*), the investigations of pharmacokinetic parameters (*Avdeeva, N.V. et al.,*

2016; Galenko-Yaroshevsky, P.A et al., 2016) clinical researches (*Shakhno, E.A et al., 2017; Filippova, O.V et al., 2015*) and modern diagnostic methods (*Tyurenkov, I.N., et al.,* 2006).

Doppler ultrasonography is a valuable diagnostic tool, which allows determining the functional state of the vasculature. The main advantage of the ultrasound method of investigation is the possibility of non-invasive assessment of the blood flow velocity in large vessels. It is known that Doppler ultrasonography does not reflect the real speed of blood flow because the obtained values depend on the angle of insonation. Consequently, when it is used this method, preference is given to the analysis of relative indicators, which is possible to calculate during carrying out the Pourcelot ratio (RI, 'the resistance index') and the Gosling pulsatility index (PI, the pulsatility index). Many researchers have shown that the Pourcelot ratio reflects the state of vascular resistance distal to the point of registration (*Zelenina, T.A., et al., 2014; Petrishchev, N.N., 2005*). However, some researchers indicate that their sensitivity varies depending on the setting of the experiment and it is applicable only to a limited range of experimental models (*Adamson SL et al., 1992*).

Objectives of the study

It is the study of the endothelium dysfunction by Doppler ultrasound examination of the velocity in the femoral artery in comparison with the changes in parameters of systemic hemodynamics and the linear velocity of microcirculatory flow of the medial broad femur muscle using laser Doppler flowmetry in conducting vasoactive pharmacological agents in intact rats and blocking the synthesis of nitric oxide rats model.

MATERIALS AND METHODS

The experiments were performed on male rats of Wistar line weighing 180-210g. The experimental animals are obtained from the Laboratory animal nursery "Stolbovaya" of Governmental Institution The Scientific Center of Biomedical Technologies Russian Academy of Medical Sciences (Moscow region). The contents and their care were carried out according to the recommendations of State Standard - 53434-2009 "Principles of good laboratory practice" International guidelines "of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" [Derective2014/63/EU]. All the experiments were approved by the local Ethics Committee (Protocol No. 7 -2017 dated 11st September 2017).

All animals were divided into two groups: 1) intact; 2) nitro-L-arginine-induced (L-NAME-induced) endothelial dysfunction (L-NAME intraperitoneally 25 mg/kg once daily for seven days) (*Pokrovskij, M.V et al., 2006)*.

A rat was anaesthetised with an intraperitoneal injection of chloral hydrate 150 mg/kg, zoletil 60 mg/kg. The right carotid artery and the right femoral neurovascular bundle were isolated, as well as the left femoral vein was catheterised.

Measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean BP was performed using a TDS160-A (Biopac, software - Acknowledge 4.2) connected to a catheter in the right carotid artery. To measure the blood flow velocity in the femoral artery, the UZOP-010-01 sensor was used with an operating frequency of 25 MHz Doppler ultrasound computerised Minimax-Doppler-K with software MM-D-K-Minimax Doppler v. 2.1 (St. Petersburg, Russia). After the sensor was installed, the following parameters were recorded above the middle third right femoral artery: maximum systolic (Vs) and diastolic velocity (Vd), mean systolic (Vas) and diastolic velocity (Vad), and the pulsatility index (PI, Gosling index) and the resistance index (RI, Pourcelot ratio). To measure the speed of movement of the shaped elements in the microcirculatory bed (linear speed of microcirculatory flow), the needle probe TDS144 (Biopac, USA) was placed in the lower third of the musculus vastus medialis. Vasoactive agents (acetylcholine 40 μg / kg, sodium nitroprusside 30 μg/kg) were inserted through a catheter placed in the left femoral vein in sequence with an interval of 15 minutes (Figure 1) (*Galagan, M.E et al., 1991; Liauder, L. Soriano et al., 2000*).

Thus, the possibility of parallel monitoring of hemodynamics and microcirculation at three levels in the system was obtained: the centre - the elastic vessel - the peripheral blood flow. Statistical processing of the results was carried out using the STATISTICA 10.0 and Microsoft Excel 2013 software package.

RESULTS

Intact rats showed the same response to the injection of acetylcholine and sodium nitroprusside, which was manifested by 1) a decrease of SBP, DBP, mean BP, linear velocity of the microcirculatory flow, Vd, Vad in the femoral artery; 2) an increase in Vs, Vas in the femoral artery, PI и RI (Fig. 1). The most of the parameters returned to their initial values for 2 minutes.

In the group of animals with L-NAME induced deficiency of nitric oxide, the response of hemodynamic parameters to after acetylcholine injection is much less pronounced than when changes investigated data after sodium nitroprusside injection (Fig. 1).

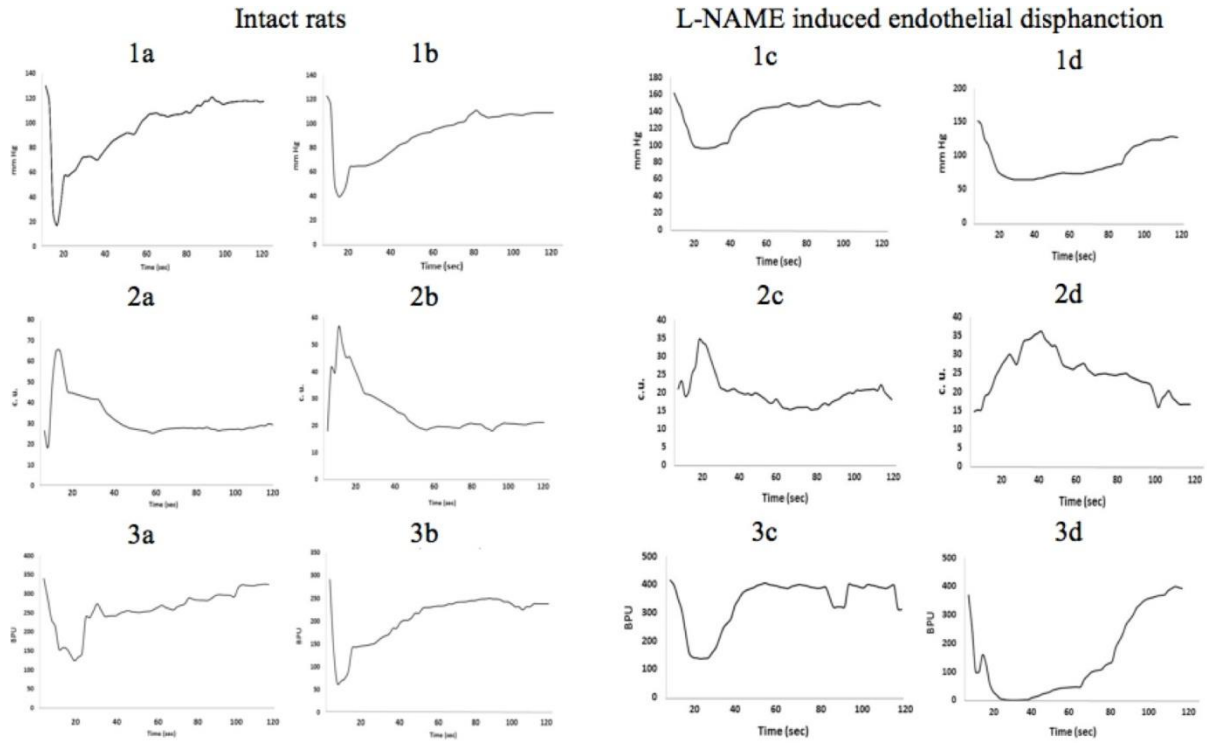

Figure 1: Endothelium-dependent (acetylcholine) and endothelium-independent (nitroprusside) vascular responses in intact rats (a, b) and in of animals with L-NAME induced deficiency of nitric oxide (c, d): the dynamics of mean BP (1a), the dynamics of the maximum systolic velocity recorded above the femoral artery (2a), dynamics of the linear velocity of microcirculation in the lower third of the medial wide thigh muscle (3a) after acetylcholine injection in intact animals; the dynamics of mean BP (1b), the dynamics of the maximum systolic velocity recorded above the femoral artery (2b), dynamics of the linear velocity of microcirculation in the lower third of the medial wide thigh muscle (3b) after sodium nitroprusside injection in intact animals; the dynamics of mean BP (1c), the dynamics of the maximum systolic velocity recorded above the femoral artery (2c), dynamics of the linear velocity of microcirculation in the lower third of the medial wide thigh muscle (3c) after acetylcholine injection in the group of animals with L-NAME induced deficiency of nitric oxide; the dynamics of mean BP (1d), the dynamics of the maximum systolic velocity recorded above the femoral artery (2d), dynamics of the linear velocity of microcirculation in the lower third of the medial wide thigh muscle (3d) after sodium nitroprusside injection in the group of animals with L-NAME induced deficiency of nitric oxide.

For statistical processing, the deviations of the values for each sample were calculated, expressed as a percentage, where the initial value was taken as 100%. Spearman correlations analysed parameters of hemodynamics. It was found, that the majority of the investigated parameters reliably correlated with each other. Taking into account that the increase in Vs and the decrease in Vd rate were recorded during the functional tests, the systolic-diastolic blood flow velocity difference (ΔVs-Vd) was included in the statistical analysis as the integrating parameters Vs and Vd and potentially more sensitive than the latter ones.

Both in the group of animals, the greatest correlation was observed between such indices as mean BP, the linear velocity of the microcirculatory flow, Vs и ΔVs–Vd ($p < 0,05$). The correlation coefficient was calculated for each animal separately, after which the mean values were calculated.

Further, the areas formed by curves of blood pressure change, microcirculation rate, Vs and ΔVs-Vd were calculated after the injection of pharmacological agents. The standard formula determined the area as the sum of the areas of rectangular trapezoids forming a figure bounded by a curve (Figure 2).

It was assessing the results of functional endothelium-independent vasodilatation (EIVD) after sodium nitroprusside injection (S_{NP}). It was calculated the area under the rising curve of the Vs and ΔVs-Vd values for the Doppler study; the area above the curve of the fall of the mean BP in the study of the parameters of central hemodynamic; area above the curve of the drop in the linear velocity of microcirculatory flow during laser Doppler flowmetry.

To study endothelium-dependent vasodilatation (EDVD) after sodium nitroprusside injection, areas

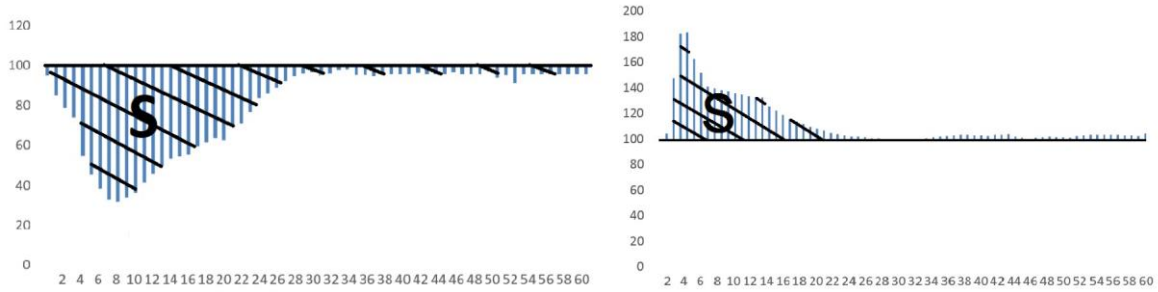

Figure 2: Example of calculating the area above the curve of the drop of the parameter; b) An example of calculating the of the parameter area under the rising curve

Table 1: The reaction areas for the tested parameters after injection of acetylcholine and sodium nitroprusside and their ratio in the group of intact rats (n = 9)

Reaction areas	Vs	ΔVs–Vd	Mean BP	the linear velocity of the microcirculatory flow
S_{AX}	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_{AX}	1.12±0.06	1.10±0.06	1.09±0.05	1.15±0.11

Table 2: The reaction areas for the tested parameters after injection of acetylcholine and sodium nitroprusside and their ratio in the group of animals with L-NAME induced deficit of nitric oxide (n = 9)

Reaction areas	Vs	ΔVs–Vd	СрАД	the linear velocity of the microcirculatory flow
S_{AX}	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_{AX}	3.94±0.27	3.99±0.29	4.05±0.31	4.11±0.52

Note: hereinafter S_{NP} - the area under the curve Vs and ΔVs-Vd and above the curve mean BP, linear velocity of the microcirculatory flow after injection of sodium nitroprusside; S_{AX} is the area under the curve Vs and ΔVs-Vd and above the curve mean BP, the linear velocity of the microcirculatory flow after injection of acetylcholine.

(S_{AX}) Were calculated under the curve of the rise in the values of Vs and ΔVs-Vd in the Doppler study; the area above the curve of the fall of the mean BP and the linear velocity of microcirculatory flow for studying the parameters of central hemodynamic and microvasculature, consequently. Then, the average S_{NP}/S_{AX} ratio was determined. The time, during which this area was calculated, was limited to 120 seconds after the injection of sodium nitroprusside and acetylcholine (Table 1). In the statistical analysis of the reliability of the differences in the S_{NP} / S_{AX}, calculated for the parameters Vs, ΔVs-Vd, mean BP and the linear velocity of microcirculatory flow, significant differences were revealed between the intact group and the group with L-NAME-induced nitric oxide deficiency for each parameter studied ($p < 0.05$).

DISCUSSION

Earlier in our laboratory to assess endothelial function, the endothelial dysfunction coefficient was proposed, which was determined by calculating the ratio of the Triangle area over the reaction curve for the recovery of mean arterial pressure after administration of nitroprusside and acetylcholine. It was 1.1 ± 0.1 in intact rats and 5.4 ± 0.6 in the group of animals with L-NAME (25 mg/kg intraperitoneally once daily for the 7 days) induced deficiency of nitric oxide (*Pokrovskij, M.V et al., 2006*).

In our study, the reactions area under the curve Vs and ΔVs-Vd, over the curve of mean BP, the linear rate of microcirculation after administration of sodium nitroprusside and acetylcholine, and their ratio in endothelium-dependent and endothelium-independent vasodilation in intact rats and the group of animals with L-NAME induced deficiency of nitric oxide. A significant difference was found between the systolic blood flow velocity and systolic-diastolic difference of blood flow velocity in the femoral artery, the linear rate of microcirculation, as well as the dynamics of mean BP between the group of intact animals and the group of animals with L-NAME induced endothelial dysfunction. In rats with endothelial dysfunction it was revealed a significant increase in the ratio of reaction areas and it was 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 rate of microcirculation, which confirms the equivalence of the research methods chosen to evaluate the function of the endothelium.

In the complex study of hemodynamic parameters and microcirculation, the greatest correlation was found between the velocity parameters of the femoral artery blood flow (Vs, ΔVs-Vd) and the dynamics of the mean BP and the linear rate of microcirculation in the group of intact animals and in animals with L- NAME induced endothelial dysfunction. The parameter ΔVs-Vd showed the greatest degree of correlation with mean BP. Perhaps, this is because the shape of the Vs curve depends more on the level of the initial BP, and Vd is more susceptible to external interference (breathing excursions, vibrations) because its absolute values are an order of magnitude less values of other parameters.

Although the Pourcelot ratio and the Gosling pulsatility index take into account the difference component of Vs and Vd, the dynamics of their change demonstrated less informative value. It is believed, that a decrease in the indices correlates with a drop in vascular resistance, but in our study, when it was performed pharmacological tests in both groups, there was an increase in these indicators. A decrease in Vd can explain the obtained data. As a result, it takes to place an increase in the numerical value of both the denominator and, to a greater extent, the numerator of the resistive and pulsatory indices determined by the ratio (Vs-Vd)/Vmean and (Vs-Vd)/Vs, respectively. This result was in agreement with the data of Adamson, 1992, and can be explained by the polar difference in the variation of these indices during local and generalised vasodilation. After systemic administration of vasodilators, the volume of circulating blood was redistributed towards the microcirculatory bed. These processes led to a drop in diastolic velocity, an increase in the amplitude of the teeth of the blood flow velocity curve, which was the causes an increase in both indices, despite a reduction in vascular resistance (*Adamson SL et al., 1992)*.

Thus, when it is performing ultrasound dopplerography, the quantitative evaluation of endothelial dysfunction with conducting vasoactive tests, it is the most indicative the calculating ratio reflecting the ratio of the areas of vascular reactions in normal and with the blockade of the synthesis of nitric oxide with the calculation of the ratio of reaction areas under the curve of the calculated index ΔVs-Vd at the EIVD to the EDVD. The obtained data are comparable with the changes in hemodynamic parameters and the linear velocity of microcirculatory flow in the musculus vastus medialis while using laser Doppler flowmetry.

SUMMARY

During calculating of S_{AX} / S_{NP} under the curve of changes in systolic blood flow velocity and systolic-diastolic difference in blood flow velocities in the femoral artery, the linear speed of microcirculation, and over the curve of the dynamics of the change in mean BP between the group of intact animals and the group of animals with L-NAME-induced endothelial dysfunction was revealed a significant difference between the studying parameters. The above confirms the possibility of applying a calculated ratio of reaction areas under the systolic-diastolic difference in blood flow velocities after sodium nitroprusside and acetylcholine administration for assessing endothelial function.

In our study it was found, that functional vascular tests both in the group of intact animals and in animals with L-NAME induced deficiency of nitric oxide were shown a high degree of correlation between changes in mean BP, systolic blood flow velocity, systolic-diastolic blood flow velocity difference in the femoral artery.

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