ORIGINAL ARTICLE

Influence of the Drug Detralex on Calcium Ion-dependent Smooth Muscle Contractility, Function of an Endothelium and Aggregation of Thrombocytes in the Conditions of Experimental Modeling of Endothelial Dysfunction

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Abstract

Introduction: The phlebotropic effect of flavonoids is contradictory, and clinically significant effects are poorly understood theoretically. The efficiency of flavonoids needs verification by means of the large-scale experimental and clinical trials protected from the conflicts of interest. **Research Tasks:** Experimental study of venotonic, anti-aggregation and endothelioprotectiv properties of the drug Detralex 1000 mg. **Materials and Methods:** A venotonic effect was investigated after week administration of drug inside on model of the endotelium-dependent smooth muscle response to increased calcium ion isolated portal vein of rats. The Biopac Bas System Station installation including a polygraph of Biopac MP 150 with the TSD-104A module and the software of ACQ 4.2 was for this purpose used. The protective properties of the endothelium were studied on a 7-day model of L-N-nitro-L-arginine-methyl ether-induced rat endothelial dysfunction using invasive blood pressure recording. Aggregation of thrombocytes (AT) was investigated by a visual micromethod with use as inductors ADF, a collagen, thrombinum, ristomycinum, and adrenaline. **Results:** In model of endothelial dysfunction rising of sensitivity to the contractile response to Ca^{2}+ the isolated segment of a portal vein after use of the drug Detralex of 500 mg/kg per day, and also elongation of AT and decrease of coefficient of endothelial dysfunction on a background the course reception of the investigated preparation. **Conclusion:** Pleiotropic effect of micronized combination of diosmin and hesperidin, expressed in strengthening the contractile potential of smooth muscle veins, improving endothelial function, and lengthening thrombocyte aggregation, was confirmed.

Key words: Detralex, diosmin, endothelial dysfunction, isolated portal vein, thrombocyte aggregation, venous insufficiency

INTRODUCTION

Diseases of the venous system are the most common pathology of peripheral vessels. Venous thromboembolic complications are an important cause of death and disability in Europe, Russia, and the USA. [1] In this regard, import substitution, creation of generics, as well as search of innovative molecules are an important task in world pharmacology. [2,3] At the same time, their studying has to be carried out on pharmacological targets,[4] *in vivo* and *in vitro* models.[5] Flavonoids - the most widespread group of the drugs which are applied at patients with a disease of peripheric veins. It is a group of biologically active substances derivative

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benzo-g-pirona, the citrus having a natural parentage and contained mainly in a peel.[6] In practical medicine, in particular, angiology gained the greatest distribution diosmin, a hesperidin, rutinum, quercetinum, and their combinations.

Diosmin has the wide range of biological effects which are confirmed with numerous researches including renders antioxidatic, anti-inflammatory, antimutagen, and antiulcerous action.[7] At the same time in a question of effective use of drugs of a diosmin, the bioavailability comes to the forefront. A number of studies have shown that micronized diosmin is superior to the rate of absorption and excretion of non-micronized analog, which led to improved clinical effect.[8]

There are data, confirmed on the basis of experiments on isolated vein segments, on the enhancement of calcium ion pressor effects by diosmin.[9] At the same time, the hesperidin and its analogs showed vasodilating effect at rats with established hypertension. A similar effect was found in flavonoid eriodictyol.[10] Follow conclusion about expediency of additional researches of flebotonic effect of flavonoids. Change of vasoconstrictor activity of the site of a portal vein of rats with the switched-off endothelial component at introduction of a diosmin in the micronized fraction, mediated calcium ion dependent the smooth muscle answer and also changes of aggregation properties of thrombocytes against the background of N-nitro-L-arginine-methyl ether (L-NAME)-model of endothelial dysfunction became a subject of our research. The selection of the venous area was based on the data on the pacemaker activity of portal vein Cajal cells, which allows to track the dynamics of the contractile response.[10]

MATERIALS AND METHODS

Experiments are drawn on rat males the Wistar lines. For the assessment of calcium ion dependent, the smooth muscle answer animals were divided into three groups, on 10 in everyone. Group 1 - control (intact rats receiving saline in equiobem doses), 2nd group - rats that received the drug Detralex 1000 mg in dose of 250 mg/kg, Group 3 - rats receiving the drug Detralex in dose of 500 mg/kg. The drug was administered 1 time a day for 7 days. For the assessment of calcium ion dependent, smooth muscle answer was carried out to samples with solutions calcium ion in concentration of 0.08–1.75 mmol.[9]

A modified Krebs-Henseleit solution was used as the base solution, in which the calcium concentration was changed, isoosmolarity was achieved by changing the sodium chloride content. Oxygenation of solutions was carried out by a mixture of gases from 95% O_2 and 5% CO_2. The drug was placed vertically in the tank of the station for testing tissues Biopac System Station (initial tension of 0.5 g). The lumen of the isolated vein was bandaged up that excluded contact of solutions to an endothelium. Registration of contractility of a vein was made by means of a polygraph of Biopac MP 150 with the TSD-104A module and the software of ACQ 4.2.

Endothelial dysfunction was modeled by means of the ADMA-similar agent - not selective blocker NO - synthase of L-NAME which was entered intraperitoneally in a dose of 25 mg/kg/days within 7 days. Hemodynamics indicators: Systolic (SBP) arterial blood pressure (ABP), diastolic arterial blood pressure (DBP), and heart rate measured continuously by means of the sensor and an apparatus complex for invasive measurement of indicators of hemodynamics of Biopac (USA) and the computer AcqKnowledge 4.2 program. Functional tests: Intravenous administration of acetylcholine (40 mg/kg) and sodium nitroprusside (30 mg/kg). Development of endothelial dysfunction in experimental animals and also extent of its correction by the studied drug estimated on the settlement coefficient of endothelial dysfunction (CED).[7]

The aggregation of thrombocytes (AT) was investigated by a visual micromethod with use as inductors ADF, a collagen, thrombinum, ristomycinum, and an adrenaline.

RESULTS AND DISCUSSION

It is established that addition of calcium ion in solution in control group led to vein tonus augmentation since concentration of 0.76 mmol/l, whereas 7-day use of Detralex increased sensitivity of a vein to calcium ion with a reliable difference from concentration of 0.25 mmol/l. Course introduction of Detralex led to reliable augmentation of sensitivity to calcium ion concentration of 0.76 mmol/l. The effect of drug has dose-dependent character that is shown in achievement of the maximum force of reduction at a larger dosage of drug in the presence of smaller concentration of calcium ion. It is most visually shown in Figure 1.

At introduction of the ADMA-similar agent - L-NAME to males within 7 days on the 8th day occurs statistically significant rising of SBP and DBP ABP with 135.7 ± 4.1 and 99.9 ± 3.3 up to 188.3 ± 6.1 and 143.0 ± 2.9 mmHg, respectively [Table 1], CED augmentation with 1.2 ± 0.1–5.0 ± 0.6 ($P < 0.05$), and depression of final metabolites of NO with 45.19 ± 2.89–22.69 ± 1.50.

Administering of the studied drug in the studied dose did not lead to statistically significant lowering of arterial pressure. At the same time, there was dose dependent statistically significant reduction of CED.

Introduction non-selective blockers NO synthase causes of disturbance of AT that is expressed in its acceleration [Table 2]. Administration of the drug Detralex brings in the expressed dose-dependent correction of disturbances that are shown in elongation of AT. It is established that in the maximum therapeutic dose, the greatest the efficiency of the drug Detralex is observed when using as the adrenaline inductor from 79.4 ± 2.7 to 96.9 ± 3.9 s.

Table 1: Influence of the drug Detralex on ABP and CED at correction of experimental dysfunction of an endothelium (M±m; *n*=10)

Group	Indicator			
	SBP mmHg	DBP mmHg	CED conventional unit	No μmol/ml
Intact	135.7±4.1^{y}	99.9±3.3^{y}	1.2±0.1^{y}	45.19±2.89 ^{y}
L-NAME	188.3±6.1*	143.0±2.9*	5.0±0.6	22.69±1.50*^{y}
L-NAME+Detralex (250 mg/кg)	185.3±4.6*	134.1±3.4*	2.4±0.4*^{y}	31.34±1.64*^{y}
L-NAME+Detralex (500 mg/кg)	174.0±4.0*	135.3±3.2*	2.0±0.1*^{y}	34.42±2.20*^{y}

P<0.05 in comparison with intact; ^{y},P<0.05 - in comparison with L-NAME. L-NAME: N-nitro-L-arginine-methyl ether, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, CED: Coefficient of endothelial dysfunction, ABP: Arterial blood pressure

Table 2: Influence of the drug Detralex on AT at correction of experimental dysfunction of an endothelium (M±m; *n*=10)

Group	Inductor			
	ADF sec.	Collagen sec.	Ristomycinum sec.	Adrenaline sec.
Intact	43.6±1.5^{y}	33.0±0.6^{y}	41.5±1.9^{y}	102.4±3.8^{y}
L-NAME	30.2±1.3*	27.1±1.1*	31.6±1.2*	79.4±2.7*
L-NAME+Detralex® 250 mg/kg	34.6±1.5*	31.5±1.0^{y}	36.2±1.5^{y}	92.3±3.9^{y}
L-NAME+Detralex® 500 mg/kg	35.2±1.4^{y}	32.1±1.0^{y}	37.2±1.36^{y}	96.9±3.9^{y}

*P<0.05 - in comparison with intact; ^{y} - P<0.05 - in comparison with L-NAME

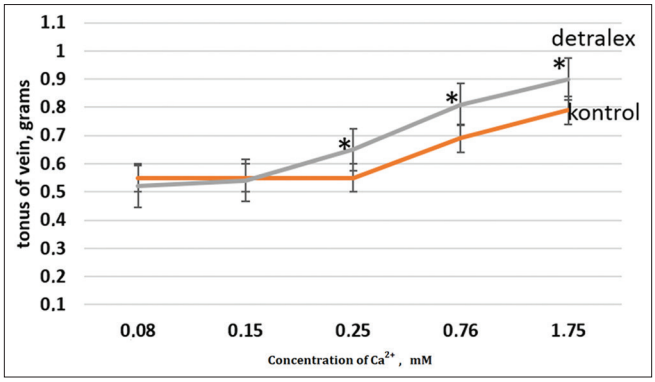

Figure 1: Influence of the drug Detralex on contractility ability of the isolated vein segment

Results of the made experiment demonstrate dose-dependent rising of sensitivity and the contractile response to calcium ion the isolated segment of a portal vein at the use of the drug Detralex of 1000 mg. It demonstrates efficiency of drug even at full dysfunction of an endothelium due to influence on calcium ion channels of smooth myocytes of a vascular wall, as causes its venotonic action.[9] Similar increase of calcium ion sensitivity of smooth myocytes is defined also under the influence of inhibitors of phosphatase[8] or agonists of alpha- adrenoreceptors receptors.[3] In a case with alpha-adrenoreceptors intensifying of sensitivity most likely is bound to G-proteins that mean the accelerated phosphorylation of a myosin component of chains.[10] Maintenance of a tonus of vessels happens as by means of phosphotazy and kinases; therefore, researches of a molecular target of a diosmin from the biochemical point of view are expedient.

The obtained data confirm efficiency of the drug Detralex of 1000 mg for correction of function of an endothelium and restoration of the NO synthesizing function at experimental endothelial dysfunction that most likely it is bound to augmentation of the activity of antioxidatic enzymes, depression of influence of endothelin-1, and a factor of a necrosis of a tumor alpha.[5] Elongation of the rate of AT in an experiment at group of the animals receiving Detralex is caused by decrease of the phenomenon of a stasis, restoration of a linear blood flow,[6] and also immediate endotelioprotectiv action.

The received results allow to recommend the drug Detralex not only as venotonic drug but also as an endotelioprotector with antiaggregant properties that are especially important at patients with comorbid pathology.

CONCLUSION

Using isolated portal vein of rats model, pleiotropic effect of micronized combination of diosmin and hesperidin, expressed in strengthening the contractile potential of smooth muscle veins, improving endothelial function, and lengthening thrombocyte aggregation, was confirmed.

REFERENCES

1. Spencer FA, Gore JM, Reed G, Lessard D, Pacifico L, Emery C, *et al*. Venous thromboembolism and bleeding in a community setting. The worcester venous thromboembolism study. Thromb Haemost 2009;101:878-85.
2. Kravchenko DV, Beskhmelnitsyna EA, Korokin MV,

Avtina TV, Sernov LN, Tishin AN, *et al*. Molecular screening of prospective candidates for TRPA1 ion channel selective antagonists. Res Result Pharmacol Clin Pharmacol 2016;2:63-6.
3. Bogus SK, Dukhanin AS, Kucheryavenko AF, Vinakov DV, Suzdalev KF, Galenko-Yaroshevsky PA. Pleyotropic antiaggregant effects of an innovative antiarrhythmic of class III SS-68, an indole derivative. Res Result Pharmacol Clin Pharmacol 2017;3:114-24.
4. Ragulina VA, Kostina DA, Dovgan AP, Burda YE, Nadezhdin SV. Nuclear factor kappa B as a potential target for pharmacological correction endothelium-associated pathology. Res Result Pharmacol Clin Pharmacol 2017;3:114-24.
5. Danilenko LM, Klochkova GN, Kizilova IV, Korokin MV. Metabolic cardioprotection: New concepts in implementation of cardioprotective effects of meldonium. Res Result Pharmacol Clin Pharmacol 2016;2:95-100.
6. Molchanova OV, Pokrovskaya TG, Povetkin SV, Reznikov KM. Endothelioprotective property of the combination of the thioctic acid and rosuvastatin shown in the endothelial dysfunction models. Res Result Pharmacol Clin Pharmacol 2016;2:9-15.
7. Feldo M, Woźniak M, Wójciak-Kosior M, Sowa I, Kot-Waśik A, Aszyk J, *et al*. Influence of diosmin treatment on the level of oxidative stress markers in patients with chronic venous insufficiency. Oxid Med Cell Longev 2018;2018:2561705.
8. Graças C, De Souza M, Cyrino FZ, De Carvalho JJ, Blanc-Guillemaud V, Bouskela E. Protective effects of micronized purified flavonoid fraction (MPFF) on a novel experimental model of chronic venous hypertension. Eur J Vasc Endovasc Surg 2018;55:694-702.
9. Savineau JP, Marthan R. Diosmin-induced increase in sensitivity to Ca^{2+} of the smooth muscle contractile apparatus in the rat isolated femoral vein. Br J Pharm 1994;111:978-80.
10. Huang X, Zhao D, Wang Z, Zhang M, Han Y, LuH, *et al*. Preliminary study on the mechanism of spontaneous rhythmic contraction in rabbit portal vein. Journal of Biomedical Engineering 2010;27:1211-5.

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