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THE EFFECTIVENESS OF TRIMETAZIDINE AND ITS COMBINATION WITH METHYLDOPA IN EXPERIMENTAL PREECLAMPSIA

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ABSTRACT

Objective of the study: To investigate the effectiveness of trimetazidine and its combination with methyldopa with preeclampsia in an experiment. **Methods:** The experiment was performed in 140 white female Wistar rats weighing 250-300 g. L-NAME was administered intraperitoneally (25 mg / kg / day) from 14 to 20 days of gestation. Trimetazidine (6 mg / kg) was administered intragastrically 1 time per day from 14 to 20 days of pregnancy. Methyldopa (2x0.043 g / kg) was administered 2 times a day from 14 to 20 days of pregnancy. On 21 days of pregnancy, functional tests and laboratory tests were performed. **Results:** The admistration in animals of trimetazidine leads to a pronounced correction of pathological changes in experimental ADMA-like preeclampsia. The greatest effect was observed with combined methyldopa. A significant decrease in systolic and diastolic pressure was noted, respectively, improved microcirculation in the placenta, restoration of the NO-synthesizing endothelial function, and a decrease in proteinuria. **Conclusion:** The results of the study indicate the promise of using trimetazidine for the correction of morphological and functional changes in preeclampsia and substantiate the feasibility of further studies in this direction.

KEYWORDS: Trimetazidine, Preeclapsia, Endothelial Dysfunction, Rats, Proteinuria, Microcirculation.

1. INTRODUCTION

About 10% of pregnancies in the world accompanied hypertensive disorders, while from 2 to 8% from preeclampsia. According to the Federal State Statistics Service, hypertensive disorders during pregnancy, birth and the postpartum period from 2013 to 2016 killed 100 women, and their prevalence in pregnant women and women in labor was 164.1 and 81.5 per 1000 births in 2013 and 2016. respectively.

In addition, hypertensive conditions during pregnancy lead to the development of pathological conditions not only in women, but also in the fetus, contributing to the disability of mothers and children [1]. The problem of preventing and treating preeclampsia, as well as assessing the severity of the course and perinatal risks, is largely due to the lack of a unified opinion of the medical community about its etiology and pathogenesis, although a huge number of studies have devoted to the study of this complication of pregnancy. Moreover, preeclampsia is increasingly seen from the point of view of endothelial dysfunction [2]. One of the mechanisms for the development of endothelial dysfunction in preeclampsia is “oxidative stress” as a result of depletion of the antioxidant system in conditions of tissue ischemia [3]. Developing against this background, endothelial dysfunction leads to impaired microcirculation and tissue hypoxia, and as a result, to the development of multiple organ disorders that make up the clinical manifestations of preeclampsia [4]. One of the leading pathophysiological factors in reducing the activity of endothelial NO-synthase (e-NOS) and the development of preeclampsia is placental ischemia. An increase in the activity of NO-synthase can be achieved by reducing ischemic phenomena of the placenta and oxidative stress due to inhibition of the enzyme 3-ketoacyl-CoA-thiolase.

Some studies have demonstrated the endothelioprotective properties of trimetazidine. Trimetazidine promotes an increase in the number of eNOS, selectively inhibiting the enzyme 3-ketoacyl-CoA-thiolase, and promotes the synthesis of nitric oxide as one of the most important factors of vasorelaxation [5]. Trimetazidine reduces the inactivation of nitric oxide by inactivating lipid peroxidation processes and protects the vascular endothelium from the direct damaging effect of free radicals and humoral factors, which indicates its anti-ischemic and antioxidant properties. Based on the foregoing, it can be assumed that the use of this drug can be effective in pregnant women with impaired growth and placenta formation in early pregnancy with outcome in placental ischemia and hypertensive disorders, the main pathogenetic mechanisms of which are severe endothelial dysfunction and oxidative stress.

Objective of the study: To investigate the effectiveness of trimetazidine and its combination with methyldopa with preeclampsia in an experiment.

2. MATERIAL AND METHODS

The experimental study was conducted at the Research Institute of Pharmacology of Living Systems of Belgorod State University. The study was performed in compliance with the requirements of General Requirements for the Competence of Testing and Calibration Laboratories 17025-2009, GOST R ISO 5725-2002 and the Rules of Laboratory Practice, approved by Order of the Ministry of Healthcare and Social Development of the Russian Federation dated August 23rd, 2010 № 708n, in compliance with the European

Convention for the Protection of Vertebrates Used for Experiments or Other Scientific Purposes CETS No. 170. All the experiments were approved by the Ethical Committee of Belgorod National Research University.

The experiment was performed in 140 white Wistar female rats weighing 250-300 g. For the formation of groups of pregnant animals with posterior periods having separate contents, males (2 animals) were planted with males (3 animals) for 24 hours. Then the animals were exposed through 10-14 days. In our experiments, pregnancy occurred in 30-40%. Then pregnant rats were divided into 5 groups:

1 group – intact;
2 group – control (administration of L-NAME);
3 group – L-NAME + Methyldopa (2 * 0,043 g/kg);
4 group – L-NAME + Trimetazidine (6 mg / kg);
5 group – L-NAME + Methyldopa (2 * 0.043 g / kg) + Trimetazidine (6 mg / kg).

ADMA-like agent - non-selective NOS inhibitor N-nitro-L-arginine-methyl Ester (L-NAME) was administered intraperitoneally at a dose of 25 mg / kg / day for seven days (14-20 days of pregnancy) [6,7]. Methyldopa (2*0.043 g / kg) was administered 2 times a day from 14 to 20 days of pregnancy. Trimetazidine (Preductal) at a dose of 6 mg / kg was administered intragastrically 1 time per day from 14 to 20 days. On the 21st day of pregnancy, the laboratory animal was anesthetized by injection intraperitonea of chloral hydrate at a dose of 300 mg / kg body weight, after which functional tests were performed [8, 9].

The degree of endothelial dysfunction in experimental animals was evaluated by the ratio of endothelium-dependent vasodilation and endothelium-independent vasodilation with subsequent calculation of the coefficient of endothelial dysfunction (CED) [10, 11, 12, 13]. The level of NO metabolites (i.e., the total concentration of nitrates and nitrites, NOx) was determined by the colorimetric method according to the development of color in the diazotization reaction of sulfonamide nitrite, which is part of the Griss reagent. To obtain data on the state of microcirculation in the placenta on the 21st day of pregnancy under anesthesia at 4 points, the microcirculation level was measured at a distance of 1 mm from the edge of the placental disc. Microcirculation values were expressed in perfusion units (PU) [14, 15]. Urine collection in intact and experimental groups of rats was carried out using special metabolic cells. The animal was placed in a cage for 12 hours with free access to water. To study the liquid content in a large oil seal, it was weighed, followed by drying at 37°C for 24 hours and repeated weighing.

Descriptive statistics were applied to all data: the data were checked for normal distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. Intergroup differences were analyzed using Student's t-test or Mann-Whitney U-test, depending on the type of distribution.

3. RESULTS AND DISCUSSION

After administration of L-NAME, a significant increase in blood pressure occurred in pregnant rats: SBP was 193.6 ± 6.28 mm Hg, DBP was 150.8 ± 80 mm Hg, while in intact animals, systolic and diastolic pressure was 123.4 ± 3.5 mm Hg. and 83.8 ± 5.47 mm Hg respectively. As a result of the administration of methyldopa (2x0.043 g / kg) and trimetazidine (6 mg / kg) per day, a significant decrease in systolic blood pressure was noted to 155.5 ± 3.40 mm Hg and 152.5 ± 1.99 mm Hg. respectively, and diastolic up to 114.4 ± 7.13 mm Hg and 112.3 ± 3.90 mm Hg, respectively (Table 1). The combined use of trimetazidine and methyldopa led to a more positive effect. Blood pressure decreased to 138.6 ± 3.10 mm Hg and 97.6 ± 5.84 mm Hg.

The administration of L-NAME to pregnant rats led to a violation of the regulatory mechanisms of vascular tone, as evidenced by an increase in CED from 1.21 ± 0.13 to 2.89 ± 0.25. The course use of methyldopa and trimetazidine for 7 days, as well as their combination in pregnant animals with ADMA-like preeclampsia, reduced CED to 2.49 ± 0.28, 1.57 ± 0.15 and 1.32 ± 0.08, respectively, which indicates an improvement in endothelial function.

In animals with ADMA-like preeclampsia, a decrease in microcirculation from 472.6 ± 22.44 PU to 215.6 ± 9.29 PU was observed. Administrations of methyldopa and trimetazidine in the studied doses restored microcirculation to 402.3 ± 15.81 PU, 402.3 ± 15.81 PU, respectively, with the greatest effect when combined (477.4 ± 27.61 PU).

Table 1: The effect of trimetazidine and its combination with methyldopa on blood pressure, CED and microcirculation in the placenta with ADMA-like preeclampsia

Indicator / Group	SBP, mm Hg	DBP, mm Hg.	CED, relative units	Microcirculation, PU
Intact	123.4±3.5*	83.8±5.47*	1.21±0.13*	472.6±22.44*
L-NAME (25 mg/kg)	193.6±6.28#	150.8±4.80#	2.89±0.25#	215.6±9.29#
L-NAME + Methyldopa (2*0.043 g/kg)	155.5±3.40*#	114.4±7.13*#	2.49±0.28#	297.8±13.41*#
L-NAME + Trimetazidine (6 mg/kg)	152.5±1.99*#	112.3±3.90*#	1.57±0.15*	402.3±15.81*#
L-NAME + Methyldopa (2*0.043 g/kg) + Trimetazidine (6 mg/kg)	138.6±3.10*#	97.6±5.84*	1.32±0.08*	477.4±27.61*

Note: SBP, DBP - systolic and diastolic blood pressure (mmHg); CED - coefficient of endothelial dysfunction (relative units); PU - perfusion units; # - p <0.05 in comparison with the group of intact animals; y * <0.05 compared with the L-NAME group.

Simulation of ADMA-like preeclampsia didn't cause significant changes in daily diuresis in pregnant rats, while at the same time it was characterized by moderate proteinuria, the values of which reached 2.34 ± 0.14 g / l (p <0.05). Administration of methyldopa, trimetazidine, and their combination from the 14th to the 20th day in pregnant animals with the simulation of ADMA-like preeclampsia significantly decreased urine protein indices compared to the control group (Table 2).

The study of the NO-synthesizing function of the endothelium was carried out on the basis of the determination of nitrite ions NOx in blood plasma from 2.20 ± 0.06 μmol / dl to 1.27 ± 0.01 μmol / dl. The administration of methyldopa, trimetazidine and their combination significantly (p <0.05) increased the content of nitrite ions (NOx) in blood plasma in animals with ADMA-like preeclampsia to 1.57 ± 0.02 μmol / dL, 1.74 ± 0.01 μmol / dl and 2.12 ± 0.02 μmol / dl, respectively.

Table 2: The effect of trimetazidine and its combination with methyldopa on diuresis and proteinuria in ADMA-like preeclampsia

Indicator / Group	Urine volume ml	Proteinuria g / l
Intact	5.40±0.22	0.85±0.07*
L-NAME (25 mg/kg)	5.63±0.21	2.34±0.14#
L-NAME + Methyldopa (2*0,043 g/kg)	5..28±0.23	1.47±0.11*#
L-NAME + Trimetazidine (6 mg/kg)	5.65±0.25	1.33±0.07*#
L-NAME + Methyldopa (2*0,043 g/kg) + Trimetazidine (6 mg/kg)	5.69±0.27	0,.98±0.14*

Note: # - p <0.05 in comparison with the group of intact animals; y * <0.05 compared with the L-NAME group.

In animals with experimental preeclampsia, an increase in the liquid content in the greater omentum was observed from 44.52 ± 0.91% to 55.44 ± 0.87%. The introduction of methyldopa, trimetazidine and their combination from day 14 to 20 in pregnant animals with the simulation of ADMA-like preeclampsia significantly reduced the fluid content in the tissues of the greater omentum to 47.95 ± 1.36%, 46.76 ± 1.74% and 45, 98 ± 2.26, respectively.

In a morphological study of the placenta in women who have undergone preeclampsia, researchers note a violation in its formation. This is manifested in the fact that there is not complete germination of the chorionic villi in the spiral arteries of the mother. At the same time, the spiral arteries retain their layers up to the muscle, which leads to trophoblast ischemia [16, 17]. The response to ischemia is the release of a large number of humoral factors, the effect of which ultimately leads to the development of endothelial dysfunction [16, 18]. In this regard, it was logical to assume that drugs with anti-ischemic action can indirectly affect endothelial dysfunction. However, the evidence that preeclampsia develops more often in women with diseases associated with impaired endothelial function and the variety of clinical variants of its manifestation does not give an exact answer to the question: "What is primary - placental ischemia or endothelial dysfunction". But it is obvious that endothelial dysfunction and placental ischemia are mutually potent components of the pathogenesis of preeclampsia.

The model of preeclampsia that we have chosen, despite the fact that it is caused by a vasoactive ADMA-like substance, also has an ischemic component. Apparently, the vessels of the placenta are most sensitive to L-NAME [19, 20], which causes their spasm and subsequent ischemia up to necrotic events [21, 22].

Inhibition of the 3-ketoacyl-CoA-thiolase enzyme can serve as a mechanism for the implementation of the protective action. A decrease in oxygen deficiency leads to a decrease in the degree of ischemia, which leads to a decrease in the release of humoral factors leading to endothelial dysfunction. In addition, inhibition of the enzyme 3-ketoacyl-CoA-thiolase leads to a decrease in the formation of peroxide radicals, which leads to a decrease in their inactivation of NO [21, 22].

The most pronounced effect is observed with the combination of trimetazidine and methyldopa. This fact is explained by the presence of a vasodilating property of the central mechanism in methyldopa. This allows you to act on various links in the pathogenesis of preeclampsia.

4. CONCLUSION

The introduction of 6 mg / kg per day of animals of trimetazidine leads to a pronounced correction of pathological changes in experimental ADMA-like preeclampsia with the greatest effect in a larger dose of the drug used. There was a significant decrease in SBP and DBP, an improvement in microcirculation in the placenta, restoration of the NO-synthesizing function of the endothelium, and a decrease in proteinuria. The most pronounced effect is observed with the combination of trimetazidine and methyldopa. The data obtained

experimentally substantiate the promise of using trimetazidine in preeclampsia and the relevance of further research in this direction.

REFERENCES

[1] Hodzhaeva ZS, Kogan EA, Klimenchenko NI et al. (2015) Clinical and pathogenetic features of early and late preeclampsia. Obstetrics and gynecology, 1, 12-17.
[2] Vodneva DN, Shmakov RG, Schegolev AI (2013) The role of trophoblast invasion markers in the development of preeclampsia and tumor progression. Obstetrics and gynecology, 11, 9-12.
[3] Krasnyj AM, Kan NE, Tyutyunnik VL et al. (2016) Oxidative stress in preeclampsia and normal pregnancy. Obstetrics and gynecology, 5, 90-95.
[4] Sidorova IS, Nikitina NA, Unanyan AL et al. (2013) Evaluation of the effectiveness of preeclampsia therapy depending on the severity of gestational endothelial dysfunction Russian Bulletin of the Obstetrician-Gynecologist, 13 (3), 4-8.
[5] Mahfoudh-Boussaid A, Hadj Ayed Tka K et al. (2014) Effects of trimetazidine on the Akt/eNOS signaling pathway and oxidative stress in an in vivo rat model of renal ischemia-reperfusion. Renal failure, 36(9), 1436-1442.
[6] Severinova OV, Lokteva TI, Gureev VV, Zhernakova NI, Osipova OA, Dolzhikov AA, Pokrovskaya TG (2019) The effect of arginase II selective inhibitors on the functional parameters of experimental animals in ADMA-like preeclampsia. Journal of International Pharmaceutical Research, 46(4), 272–275.
[7] Gureev VV, Alehin SA, Pokrovskiy MV, Dolghikov AA, Korokin MV, Gudyrev OS, Kolesnik IM (2014) Remote ischemic preconditioning correction in ADMA-like gestosis model. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(5), 1095–1098.
[8] Gureev VV, Pokrovskii MV, Korokin MV, Gudyrev OS, Philippova OV, Dolzhikov AA, Lazareva GA (2015) Correction of ADMA-induced preeclampsia with use of tetrahydrobiopterin and selective inhibitor of arginase II ZB49-0010. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(5), 1538–1541.
[9] Severinova OV, Gureev VV, Zhilinkova LA, Lazareva GA, Gureeva AV, Lazareva SS (2019) Study of the effect of selective inhibitor of Arginase II KUD 975 and of low doses of Acetylsalicylic acid on the functional parameters of the cardiovascular system In experimental preeclampsia. Research Results in Pharmacology 5(4), 47–56.
[10] Korokin MV, Soldatov VO, Tietze AA, Golubev MV, Belykh AE, Kubekina MV, Puchenkova OA, Denisyuk TA, Gureyev VV, Pokrovskaya TG, Gudyrev OS, Zhuchenko MA, Zatolokina MA, Pokrovskiy MV (2019) 11-amino acid peptide imitating the structure of erythropoietin α-helix b improves endothelial function, but stimulates thrombosis in rats. Pharmacy & Pharmacology.;7(6):312-320.
[11] Pokrovskii MV, Korokin MV, Kudryavtsev KV, Pokrovskaya TG, Gudyrev OS, Gureev VV, Korokina LV, Povetkin SV (2017) Study of endothelial protective activity of phenol-derived thrombin and arginase-2 inhibitors KUD-259 and KUD-974. Bulletin of Experimental Biology and Medicine, 163(4), 436-438. doi:10.1007/s10517-017-3822-y
[12] Chernomortseva ES, Pokrovskiĭ MV, Pokrovskaia TG, Artiushkova EV, & Gureev VV (2009) Experimental study of cardioprotective and endothelioprotective action of macrolides and azalides. Experimental and Clinical Pharmacology, 72(2), 29-31.
[13] Stupakova EG, Lazareva GA, Gureev VV (2018) Correction of morphofunctional disturbances arising when modelling Preeclampsia with resveratrol and nicorandil. Research Results in Pharmacology 4(1), 59-71.
[14] Korokin MV, Pokrovskii MV, Gudyrev OS, Korokina LV, Pokrovskaia TG, Lazarev AI, Philippenko NG, Gureev VV (2015) Pharmacological correction of endothelial dysfunction in rats using e-NOS cofactors. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(5), 1548–1552.
[15] Ducray JF, Naicker T, Moodley J (2011) Pilot study of comparative placental morphometry in preeclamptic and normotensive pregnancies suggests possible maladaptations of the fetal component of the placenta. European Journal of Obstetrics & Gynecology and Reproductive Biology, 156(1), 29-34.
[16] 17. Fang MR, Li JC (2005) Evaluation of the efficacy of ligustrazine collaborated with magnesium sulfate in the treatment of pregnancy-induced hypertension in rats. Shi Yan Sheng Wu Xue Bao, Journal of experimental biology, 38(1), 45-53.
[17] Sheppard SJ, Khalil RA (2010) Risk Factors and Mediators of the Vascular Dysfunction Associated with Hypertension in Pregnancy. Cardiovascular & Hematological Disorders-Drug Targets, 10(1), 33-52.
[18] Grill S, Rusterholz C, Zanetti-Dällenbach R et al. (2009) Potential markers of preeclampsia. Reproductive Biology and Endocrinology, 14(7), 70.
[19] Wang QJ, Song BF, Zhang YH (2015) Expression of RGC32 in human normal and preeclamptic placentas and its role in trophoblast cell invasion and migration. Placenta, 36(4), 350-356.
[20] Rosselli M, Keller PJ, Dubey RK (1998) Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Human Reproduction Update, 4(1), 3-24.
[21] Ruiqiong MA, Sun Minna, Yang Zi (2010) Effects of preeclampsia-like symptoms at early gestational stage on feto-placental outcomes in a mouse model. Chinese Medical Journal, 123(6), 707-712.