Research Article

The effect of acetylsalicylic acid and its combination with methyldopa on the functional parameters of rats in asymmetric dimethylarginine-like preeclampsia

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ABSTRACT

Introduction: Preeclampsia is still one of the most common causes of maternal and perinatal mortality worldwide. Violations in the hemostatic system observed in preeclampsia can be its consequence, as well as independently exacerbate existing violations, which entails the development of a more pronounced generalized vascular spasm, increased blood pressure and, as a consequence, ischemic disorders in the organs. **Objective:** The objective of the study was to the protective activity of a small dose of acetylsalicylic acid (ASA) and its combination with methyldopa in the correction of asymmetric dimethylarginine (ADMA)-like preeclampsia. **Materials and Methods:** The study was performed on 150 female Wistar rats weighing 250-300 g. **Research Results:** During the series of experiments, the expressed protective activity of ASA in combination with methyldopa against morphofunctional disorders in ADMA-like preeclampsia was established. **Conclusion:** The combination of ASA and methyldopa is a promising direction in the treatment of preeclampsia in pregnant women.

KEY WORDS: Acetylsalicylic acid, Ischemic disorders, Methyldopa

INTRODUCTION

Hypertensive disorders during pregnancy are still one of the most common causes of maternal and perinatal mortality worldwide. It is estimated that 2–8% of pregnancies worldwide are complicated by preeclampsia.[1-3] In recent years, the number of studies indicating that the main role in the development of preeclampsia plays a violation of the functional state of the vascular endothelium, which entails the development of generalized spasm, increased blood pressure, and, as a consequence, ischemic organ damage.[4-6] The endothelium is a powerful endocrine organ involved in the regulation of vascular tone, rheological properties of blood, and processes of local inflammation. Normally, anticoagulant and antithrombotic activity of the endothelium prevail over procoagulant properties, but in preeclampsia this balance shifts toward procoagulation.[7] At present, there is increasing evidence that changes in angiogenic factors and coagulopathy are responsible for the clinical manifestations of this condition. The mechanism of triggering the vascular-platelet hemostasis in case of vessel damage consists of multistage activation of platelets in interaction with subendothelial cells.[8] This process occurs in other conditions and becomes generalized with the development of preeclampsia. The process of interaction of platelets and endothelial cells in the pathogenesis of preeclampsia still requires further study, but their union in a separate link within hemostasis emphasizes their close relationship. Activation of the coagulation process in contact with platelets damaged endothelium leads to an increased expenditure, as well as to the formation of platelets in the bone marrow.[9,10] Platelet contact with the damaged endothelium may represent the initial stage of the coagulation cascade, which leads to an increase in platelet consumption in the uteroplacental bed followed by a decrease in the number of circulating platelets in the first phase of the process. In this case, activated platelets are used locally and practically do not enter the systemic circulation.[11]

In preeclampsia, endothelial dysfunction as well as disorders in the hemostatic system are formed at

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the preclinical stage of this complication. Platelets and endothelial cells, which provoke inflammatory response and hemocoagulation at the microcirculatory level, are the main provoking links of the immunoinflammatory cascade. From the point of view of the preeclampsia and placental insufficiency formation mechanisms, it is pathogenetically reasonable to take low doses of acetylsalicylic acid (ASA) in high-risk pregnant women for prophylactic purposes.[12-15]

The use of low-dose aspirin is recommended for women with risk factors for preeclampsia to reduce morbidity and mortality associated with this complication.[16-19] The favorable effect of aspirin on the courses of preeclampsia, due to the influence of ASA on platelet activity. By inhibiting the synthesis of thromboxane A 2, aspirin reduces platelet activation.[20] Understanding the pharmacokinetic and pharmacodynamic features of this drug create prerequisites for the study of the possibility of correcting morphofunctional disorders arising from experimental preeclampsia.

Objective

The objective of the study was to the protective activity of a small dose of ASA and its combination with methyldopa in the correction of asymmetric dimethylarginine (ADMA)-like preeclampsia.

MATERIALS AND METHODS

The study was performed on 150 female Wistar rats weighing 250–300 g. An ADMA-like model of preeclampsia was reproduced in the experiment by introducing a nonselective endothelial nitric oxide synthase (eNOS) blocker of N-nitro-L-arginine methyl ether (L-NAME) 25 mg/kg/day intraperitoneal from the 14th to the 20th day of pregnancy.

Groups of pregnant animals were obtained by transplanting males (2 animals) to females (3 animals) for a day and then the animals were seated. The fact of pregnancy was established on the 10–14 day by palpation of the anterior abdominal wall in a drug-induced sleep.

Table 1: Results of ADMA-like preeclampsia correction with a small dose of ASA and in combination with methyldopa in rats (M±m; *n*=10)

Group	Indicator			
	SBP (mm Hg)	SBP (mm Hg)	CED (conv.)	Microcirculation (PU)
Intact animals	127.6±1.5^{y}	91.9±5.63^{y}	1.32±0.8^{y}	487.9±22.56^{y}
L-NAME	200.5±6.32*	151±5.69*	3.13±0.21*	210.2±11.18*
L-NAME+methyldopa 0.043 g/kg×2 t/day	138.8±3.27*^{y}	99.4±4.36^{y}	2.53±0.18*^{y}	275.3±15.71*^{y}
L-NAME+ASA 50 mg/kg/day	192.3±7.62*	146.5±4.19*	1.78±0.11*^{y}	414±14.7*^{y}
L-NAME+ASA 50 mg/kg/day+methyldopa 0.043 g/kg×2 t/day	131.0±2.0^{y}	92.4±2.8^{y}	1.51±0.1^{y}	469.8±15.44^{y}

*P<0.05 compared to the intact animal group; ^{y}P<0.05 compared to the L-NAME group. ADMA: Asymmetric dimethylarginine, ASA: Acetylsalicylic acid, L-NAME: N-nitro-L-arginine methyl ether, SBP: Systolic blood pressure, CED: Coefficient of endothelial dysfunction

Table 2: Evaluation of functional parameters in rats with ADMA-like preeclampsia after administration of the ASA and its combination with methyldopa small dose (M±m; *n*=10)

Group	Indicator		
	NO (µmol/l)	Proteinuria (g/l)	Omentum edema (%)
Intact animals	2.28±0.05^{y}	0.23±0.051^{y}	44.39±1.62^{y}
L-NAME	1,3±0.02*	2.2±0.177*	55.02±1.74*
L-NAME+methyldopa 0.043 g/kg×2 t/day	1.54±0.04*^{y}	1.22±0.103*^{y}	49.43±1.64*^{y}
L-NAME+ASA 50 mg/kg/day	1.75±0.03*^{y}	0.86±0.147*^{y}	49.33±1.94^{y}
L-NAME+ASA 50 mg/kg/day+methyldopa 0.043 g/kg×2 t/day	2.12±0.03^{y}	0.33±0.09^{y}	44.32±2.1^{y}

*P<0.05 compared to the intact animal group, ^{y}P<0.05 compared to the L-NAME group. ADMA: Asymmetric dimethylarginine, ASA: Acetylsalicylic acid, L-NAME: N-nitro-L-arginine methyl ether, NO: Nitric oxide

Table 3: Effect of ASA and its combination with methyldopa on fetal growth and weight indices in ADMA-like preeclampsia in rats (M±m; *n*=10)

Indicator group	Fetal mass, g	Fetal growth, mm	Growth/mass, mm/g
Intact animals	1.56±0.03^{y}	23.00±0.47	14.77±0.15^{y}
L-NAME	1.44±0.03*	23.3±0.45	16.22±0.13*
L-NAME+methyldopa 0.043 g/kg×2 t/day	1.53±0.03^{y}	24.05±0.41	15.74±0.16^{y}*
L-NAME+ASA 50 mg/kg/day	1.54±0.03^{y}	23.75±0.48	15.47±0.13^{y}*
L-NAME+ASA 50 mg/kg/day+methyldopa 0.043 g/kg×2 t/day	1.6±0.04^{y}	23.65±0.54	17.86±0.11^{y}

*P<0.05 compared to the intact animal group, ^{y}P<0.05 compared to the L-NAME group. ADMA: Asymmetric dimethylarginine, ASA: Acetylsalicylic acid, L-NAME: N-nitro-L-arginine methyl ether

Then, the pregnant rats were randomized into five groups:

- 1st control group – intact (animals with physiologically proceeding pregnancy)
- 2nd control group – animals with ADMA-like preeclampsia that were administered a nonselective blocker NOS L-NAME (25 mg/kg/day) intraperitoneal from the 14th to the 20th day of pregnancy)
- 3rd group-L-NAME + methyldopa which was administered in dose 0.043 g/kg 2 times a day orally from 14th to 20th days of pregnancy
- 4th group-L-NAME + ASA at a dose of 50 mg/kg/day orally from 14th to 20th days of pregnancy
- 5th-L-NAME + ASA at a dose of 50 mg/kg/day + methyldopa at a dose of 0.043 g/kg/day twice a day from the 14th to the 20th day of pregnancy route of administration orally. On the 21st day of gestation, the experimental animal was anesthetized by intraperitoneal injection of chloral hydrate at a dose of 300 mg/kg body weight and after that functional tests were performed.

RESULTS

The administration of ASA (50 mg/kg/day) in animals with ADMA-like preeclampsia revealed a statistically significant ($P < 0.05$) decrease in blood pressure in relation to the group of "untreated" animals. However, with the administration of a combination of ASA and methyldopa, this indicator almost reached the level of animals with physiologically proceeding pregnancy [Table 1].

Assessing the results of the vascular endothelium functional state indicators, a statistically significant ($P < 0.05$) improvement in the coefficient of endothelial dysfunction of animals with ADMA-like preeclampsia, who were administered ASA in relation to the group of "untreated" animals, was revealed. With the introduction of the ASA and methyldopa combination, the coefficient of endothelial dysfunction almost reached the level of intact animals [Table 1].

Assessing the level of nitric oxide final metabolites concentration in rats' plasma with ADMA-like preeclampsia was revealed statistically significant ($P < 0.05$) in relation to the group of "untreated" animals increase in the groups of animals with ADMA-like preeclampsia, which was administered ASA in combination with methyldopa, while using the studied pharmacological agents as monotherapy, this indicator was less pronounced [Table 2].

The administration of ASA, as well as methyldopa to animals with experimental preeclampsia had a statistically significant ($P < 0.05$) decrease in the concentration of protein in the urine of experimental animals, but the combined administration of the studied pharmacological agents reduced proteinuria almost to the level of animals with physiological pregnancy [Table 2].

The study of the fluid content in the tissues of the greater omentum in animals with experimental preeclampsia revealed a statistically significant ($P < 0.05$) decrease in this indicator with the combined administration of ASA and methyldopa in comparison with the group of "untreated" animals and the achievement of its level of intact animals [Table 2].

The study of the correction of height and weight parameters results in fetuses with the experimental preeclampsia showed an increase in body weight of fetuses in the group with combined ASA with methyldopa [Table 3]. It should be noted that the ratio of growth and weight of the fetus approached the level of statistically indistinguishable from the level of intact animals.

CONCLUSION

Summarizing all the above, it can be argued that the results of the series of experiments indicate the most pronounced protective activity of ASA in combination with methyldopa in the correction of morphofunctional disorders in ADMA-like preeclampsia. That can be explained by the fact that the combined use of the studied drugs affects various key points of preeclampsia. Apparently, the reduction of blood pressure due to central mechanisms and correction of functional interaction in the platelet-endothelium system is accompanied by a positive summation of effects.

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