Study of the effect of acetylsalicylic acid and a selective arginase II inhibitor KUD 975 on the correction of hemostatic disorders in experimental preeclampsia

Anastasia V. Gureeva1, Olga V. Severinova2, Vladimir V. Gureev2, Indira S. Kochkarova2, Elena V. Avdeyeva1

1 Kursk State Medical University, 3 K. Marx St., Kursk 305041, Russia
2 Belgorod State National Research University, 85 Pobedy St., Belgorod 308015, Russia

Corresponding author: Olga V. Severinova (frendic@mail.ru)

Academic editor: Mikhail Korokin
Received 8 April 2022
Accepted 29 June 2022
Published 12 July 2022

Citation: Gureeva AV, Severinova OV, Gureev VV, Kochkarova IS, Avdeyeva EV (2022) Study of the effect of acetylsalicylic acid and a selective arginase II inhibitor KUD 975 on the correction of hemostatic disorders in experimental preeclampsia. Research Results in Pharmacology 8(3): 1–8. https://doi.org/10.3897/rrpharmacology.8.87539

Abstract

Introduction: The disruption of the functional state of the vascular endothelium is among the main causes of preeclampsia, which is one of the most common causes of maternal and perinatal mortality. It can be enhanced by the humoral factors secreted by the activated platelets. The use of acetylsalicylic acid is an effective way to prevent preeclampsia. However, its ability to activate eNOS is a prerequisite for researching its ability to correct the disorders in developing preeclampsia, including by reducing the platelet activity. In this case its effect can be enhanced through increasing the bioavailability of L-arginine by using a selective arginase II inhibitor KUD 975. These facts were the prerequisite for conducting this study.

Materials and methods: The study was conducted on 180 female Wistar rats weighing 250–300 g. Acetylsalicylic acid was used at a dose of 7 mg/kg/day and 10 mg/kg/day, KUD 975 – at a dose of 1 mg/kg/day and 3 mg/kg/day. Adenosine diphosphate (ADP, 6.5 microns), arachidonic acid (ASPI, 0.5 mM), and collagen (3.2 mcg/ml) were used as aggregation inducers.

Results and discussion: ADMA-like preeclampsia simulation led to an increase in platelet aggregation ability when using all aggregation inducers. This is evidenced by an increase in a degree, rate of aggregation, and a shortened time of thrombus formation. The use of acetylsalicylic acid and a selective arginase II inhibitor KUD 975 led to a decrease in the aggregation ability of platelets and an increase in thrombosis time, while the combined administration of the studied agents showed a more pronounced effect.

Conclusion: The data obtained while performing a series of experiments strongly indicate a promising outlook for using acetylsalicylic acid and a selective arginase II inhibitor KUD 975 in order to correct emerging disorders in preeclampsia.

Keywords

selective arginase II inhibitor KUD 975, acetylsalicylic acid, preeclampsia, platelets.
Introduction

Preeclampsia currently remains an urgent problem of the modern obstetrics, as this complication is one of the most common causes of maternal and perinatal mortality worldwide. The frequency of this complication ranges from 2 to 8% of pregnancies (Gureev et al. 2014; Adamyan et al. 2016; Yakushev et al. 2016). The recent studies have named a dysfunction of the functional state of the vascular endothelium among the main causes of the development of preeclampsia, which entails the development of the generalized vascular spasm, an increase in blood pressure, as well as disorders in the hemostasis system and, as a consequence, ischemic disorders in organs (Nguyen et al. 2016; Lukyanova et al. 2018).

The process of interaction of platelets and endothelial cells in the pathogenesis of preeclampsia still requires further study, but their association into a separate element within hemostasis emphasizes their close connection (Gureev et al. 2015; Kohli et al. 2016). Activation of maternal platelets or the dysfunction of endothelial cells during pregnancy can initiate a cascade of events that lead to preeclampsia. Platelet activation can not only be caused by the proteins of subendothelial structures, but also by adrenaline, noradrenaline, ADP, immune complexes, peroxide radicals, hypoxia, arterial hypertension, prostaglandins PG2 and PGH2, arachidonic acid, thromboxane A2, platelet activation factor (FAT), forbol esters, latex, lectins, ionophore A23187, bacteria and bacterial lipopolysaccharide, tumor cells, increased shear stress, etc. The placenta in ischemic conditions secretes many of these humoral factors. Thus, platelet activation can be considered as an intermediate pathogenic link between the immaturity of spiral arteries, placental ischemia and systemic endothelial dysfunction (Tannetta et al. 2015; Kohli et al. 2016; Jacobsen et al. 2019; Pickel et al. 2019).

The contact of platelets 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. There is evidence in the literature which indicates that the platelet production time in hypertension in pregnant women is significantly reduced when compared to a normal pregnancy (Gardiner and Vatish 2017; Kohli and Isermann 2017). Subsequently, there may be a compensatory increase in platelet production by the bone marrow (Kohli and Isermann 2017). Young platelets released into the bloodstream have a higher tendency to aggregate.

Currently, the use of low doses of acetylsalicylic acid is recognized as the only most effective method of prevention; however, the dosage regimen of this drug remains a matter of dispute for scientists around the world. For example, a number of international recommendations, including WHO recommendations, state that after 12 weeks of pregnancy, the appointment of ASA at a dose of 75–100 mg per day is required (Dzeshka et al. 2016; Estevez and Du 2017). However, studies have demonstrated the effectiveness of this method of prevention only for women belonging to the high-risk group for the development of preeclampsia, where acetylsalicylic acid reduced the risk of developing an early and severe preeclampsia (Yakushev et al. 2012; Dzeshka et al. 2016). More recent studies have concluded that the dose of acetylsalicylic acid should be more than 100 mg per day and prescribing it after 16 weeks of pregnancy has more benefits for the prevention of preeclampsia (Dzugkoev et al. 2018; Khodzhaeva et al. 2018; Netrebenko et al. 2021).

Another positive property of acetylsalicylic acid is its ability to acetylate lysine eNOS. It leads to its activation and increased synthesis of NO, not only in endothelial cells, but also in platelets. NO is not only a powerful vasodilator, but also a factor preventing the platelet activation (Estevez and Du 2017). Moreover, this effect turned out to be independent of the inhibition of COX-1 and the production of ThA2 (Dzeshka et al. 2016). Thus, the presented data indicate the possibility of using acetylsalicylic acid not only for preventive purposes, but also for therapeutic purposes in preeclampsia.

A logical way to enhance the action of acetylsalicylic acid is to increase the bioavailability of L-arginine. This can be achieved by using selective arginase II inhibitors. In particular, the combination under the laboratory cipher KUD-975. Despite the above, there is no data in the modern literature on the effect of acetylsalicylic acid and a selective arginase II inhibitor on the aggregation ability of platelets in the condition of ADMA-like preeclampsia. This circumstance was the reason for the present study.

The aim of the study

To conduct a study of the hemostasis system in the condition of ADMA-like preeclampsia and the possibility of correcting its disorders with acetylsalicylic acid, arginase II inhibitor KUD 975 and their combined administration.

Materials and methods

Compounds under study

A compound of phenolic nature is a selective arginase II inhibitor a substance with the laboratory code KUD 975.

Figure 1. Methyl ether (2-((1-hydroxynaphthalene-2-yl) thio) acetyl)-D-proline (C18H19NO4S).
Animals

The study was conducted on 180 female Wistar rats weighing 250–300 g. The experimental study was conducted on the basis of the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University (BelSU). The animals were kept in individually ventilated "Tecniplast" cells for small laboratory animals. UV-sterilized sawdust was used as bedding. The feed was pelleted diet for small laboratory animals (rodents). Water was purified and sterilized by UV irradiation. The microclimate was created and maintained by a system of individually ventilated cells. Acclimatization and selection of animals for the study involved at least a 10-day quarantine. The animals were divided into groups by body weight. The animals were marked by labeling their bodies. At the time of the study, the animals were healthy, without changes in behavior, appetite, and sleep-and-wake schedule. For 18 hours before the experiments, the animals had been completely deprived of food, with free access to water.

All the manipulations on experimental animals were performed under general anesthesia, performed by intraperitoneal administration of chloral hydrate. Experimental studies were approved by the Bioethical Committee of Belgorod State National Research University (Minutes No. 1/19 of 16.01.2019). Vivisection was performed in accordance with the ethical principles of treating laboratory animals according to “The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. CETS № 123”.

Experiment design

The males (2 animals) were put into the cage with the females (3 animals) for a day in order to form groups of pregnant animals in conditions of separate maintenance. Then the animals were separated, and 10–14 days later, in the conditions of drug-induced sleep, the fact of pregnancy was established by palpating the anterior abdominal wall.

After that the pregnant rats were randomized into 11 groups:

• Group 1 – intact (animals with a physiologically proceeding pregnancy);
• Group 2 – control (simulation of ADMA-like pre-eclampsia in the studied animals was performed by introducing a non-selective NOS blocker N-nitro-L-arginine-methyl ether (L-NAME) 25 mg/kg/day intraperitoneally from the 14th to the 20th day of pregnancy);
• Group 3 – L-NAME + L-norvaline at a dose of 10 mg/kg/day from the 14th to the 20th day of pregnancy (oral administration);
• Group 4 – L-NAME + methyldopa at a dose of 0.043 g/kg/day × 2 r/day from the 14th to the 20th day of pregnancy (oral administration);
• Group 5 – L-NAME + selective arginase II inhibitor, KUD 975 at a dose of 1 mg/kg/day orally from the 14th to the 20th day of pregnancy;
• Group 6 – L-NAME + selective arginase II inhibitor, KUD 975 at a dose of 3 mg/kg/day orally from the 14th to the 20th day of pregnancy;
• Group 7 – L-NAME + acetylsalicylic acid at a dose of 7 mg/kg/day orally from the 14th to the 20th day of pregnancy;
• Group 8 – L-NAME + acetylsalicylic acid at a dose of 10 mg/kg/day orally from the 14th to the 20th day of pregnancy;
• Group 9 – L-NAME + selective arginase II inhibitor, KUD 975 at a dose of 3 mg/kg/day orally + acetylsalicylic acid at a dose of 10 mg/kg/day orally from the 14th to the 20th day of pregnancy;

On the 21st day of gestation, the experimental animal was anesthetized by intraperitoneal injection of chloral hydrate at a dose of 300 mg/kg of body weight, after which functional tests were performed.

Functional tests

The study of platelet aggregation function was carried out in the whole blood by an impedance method, according to the manufacturer’s instructions, on an automatic aggregometer Multiplate (Verum Diagnostica, Germany). After the standard setup of Multiplayer 2.0, 300 µl of NaCl was loaded into the cuvettes. Then 300 µl of the whole blood was injected into each cuvette, followed by a three-minute incubation with an automatic mixing of the sample. After the incubation, 20 µl of agonist was added to each corresponding cuvette. Adenosine diphosphate (ADP, 6.5 µm), arachidonic acid (ASPI, 0.5 mM), collagen (3.2 mcg/ml) were used as the aggregation inducers. The platelet aggregation was measured by electrical impedance. The measurements included each of the following parameters: platelet aggregation capacity (AU (aggregation units)), platelet aggregation rate (AU/min), area under the curve (AUC), measured in U=AU*min (1U=10AU*min). Coagulogram parameters were determined on a Minilab 701 device, using reagents for determining prothrombin time (PTT), thrombin time (TT), activated partial thromboplastin time (APTT), and fibrinogen test (manufactured by SPD RENAM, Russia).

The time of thrombus formation was determined on the 20th day of pregnancy in anesthetized (chloral hydrate 400 mg/kg) female rats with EG. Thrombosis was caused by the application of a 50% solution of ferric chloride (III); for this reason, the area of the isolated carotid artery was isolated from the surrounding tissues, and a cotton pad moistened with a 50% solution of ferric chloride

![Acetylsalicylic acid (C9H8O4).](image-url)
(0.025 ml) was placed on it. Blood flow was recorded above the application site using a Doppler sensor (Minimax – Doppler-K, St. Petersburg, Russia). The time of thrombus formation was measured from the moment of application of the iron (III) chloride solution to the complete cessation of blood flow in the carotid artery (Kurz 1990).

**Statistical data processing**

Descriptive statistics was applied to all the data: the data were checked for the normality of distribution. The type of distribution was determined by the Shapiro-Wilk criterion. In the case of a normal distribution, the mean (M) and the standard error of the mean (m) were calculated. The intergroup differences were analyzed by parametric (Student’s t-test) or nonparametric (Mann-Whitney test) methods, depending on a type of distribution. The statistical significance of differences between morphological changes after their ranking was assessed using the Mann-Whitney nonparametric data analysis method. The calculations were performed using the Microsoft Excel 7.0 statistical software package, the GraphPad Prism 8 editor was used to create illustrations.

**Results and discussion**

An increase in platelet aggregation ability is observed when modeling ADMA-like preeclampsia by 7-day administration of L-NAME. This is evidenced by a statistically significant (p<0.05) increase in platelet aggregation, the area under the platelet aggregation curve and platelet aggregation rate when using ADP as an aggregation inducer by 76.4±4.38%, 67.4±5.62% and 75.4±4.84% compared to those in the group of intact animals, respectively. Using collagen as an inducer, the platelet aggregation increased by 95.6±4.50% compared to that in the group of intact animals, the area under the platelet aggregation curve increased by 89.9±4.71%, and platelet aggregation accelerated by 86.5±4.48%. Using arachidonic acid, an increase in these indicators compared to the group of intact animals occurred by 80.5±4.21%, 81.0±4.49% and 81.7±5.04%, respectively.

There was no statistically significant increase in the aggregation ability in relation to the group of intact animals with the introduction of 10 mg of acetylsalicylic acid. When using KUD 975 at a dose of 1 mg in animals with ADMA-like preeclampsia, the level of platelet aggregation ability compared to that in the group of intact animals was 49.0±6.23% higher, and when using KUD 975 at a dose of 3 mg, it was 23.3±2.79% higher (Fig. 3A), which statistically significantly differed from that in the group of “untreated” animals (p<0.05).

When using collagen as an inducer of platelet aggregation against the background of administration of acetylsalicylic acid at a dose of 7 mg, an increase in aggregation ability was noted in the animals with ADMA-like preeclampsia by 41.6±6.74% when compared to the group of intact animals, which is statistically significant (p<0.05) to the group of “untreated” animals (95.6±4.50%), and with the introduction of acetylsalicylic acid at a dose of 10 mg, this indicator increased only by 12.4±5.41%, which was comparable to the group of intact animals. Against the background of the introduction of KUD 975 at a dose of 1 mg, the aggregation capacity of platelets increased by 59.4±6.14%, and at a dose of 3 mg – by 34.4±5.54% compared with that in the group of intact animals and statistically significantly (p<0.05) differed from that in the group of “untreated” animals (Fig. 3B).

The study of platelet aggregation when using arachidonic acid as an inducer showed an increase in this indicator in the animals with ADMA-like preeclampsia against the background of administration of acetylsalicylic acid at a dose of 7 mg by 34.1±5.79%, which statistically significantly (p<0.05) differed from that in the group of “untreated” animals (80.5±4.21%), and when the acetylsalicylic acid was administered at a dose of 10 mg, this indicator increased by 2.1±4.27%, which is comparable to the indicators of intact animals. Against the background of the introduction of KUD 975 at a dose of 1 mg, the aggregation capacity of platelets increased by 48.3±6.24%, and at a dose of 3 mg – by 25.6±2.89% when compared to that in the group of intact animals and statistically significantly (p<0.05) differed from that in the group of “untreated” animals (Fig. 3D).

When assessing the rate of platelet aggregation under conditions of using ADP as an inducer against the background of the introduction of acetylsalicylic acid at a dose of 7 mg, this indicator increased by 24.8±6.62%, which statistically significantly (p<0.05) differed from that in the group of “untreated” animals (67.4±5.62%), and with the introduction of acetylsalicylic acid at a dose of 10 mg – decreased below the level of intact animals by 4.0±4.48%. With the introduction of KUD 975 at a dose of 1 mg, the platelet aggregation rate increased by 38.6±8.00%, and at a dose of 3 mg – by 14.7±4.24% when compared to that in the group of intact animals (Fig. 3B).

In collagen-induced conditions, against the background of the introduction of acetylsalicylic acid at a dose of 7 mg, the platelet aggregation rate increased by 41.5±5.06%, which statistically significantly (p<0.05) differed from that in the group of “untreated” animals (86.5±4.48%), and with the introduction of acetylsalicylic acid at a dose of 10 mg – to 9.0±5.42% when compared to the group of intact animals. When KUD 975 was administered at a dose of 1 mg, the platelet aggregation rate increased by 61.0±4.93%, and at a dose of 3 mg – by 36.0±3.71% when compared to that in the group of intact animals, which statistically significantly differs from the level of “untreated” animals (Fig. 3G).

The induction of platelet aggregation with arachidonic acid resulted in an increase in the platelet aggregation rate with the introduction of acetylsalicylic acid at a dose of 7 mg by 37.6±7.14%, which statistically significantly (p<0.05) differed from that in the group of “untreated” animals (81.7±5.04%), and with the introduction of acetylsalicylic acid at a dose of 10 mg – by only 7.0±3.76%
when compared to that in the group of intact animals. With the introduction of KUD 975 at a dose of 1 mg, the platelet aggregation rate increased by 45.9±6.58%, and at a dose of 3 mg – by 25.4±4.60% when compared to that in the group of intact animals, which statistically significantly (p<0.05) differed from the group of “untreated” animals (Fig. 3E).

The results of the study of the area under the platelet aggregation curve during the induction by ADP, collagen, and arachidonic acid were used as inducers, demonstrated a sharp decrease in these indicators by 12.9±5.9%, 5.8±6.2%, 11.3±7.0% and by 19.0±6.7%; 8.5±3.1%; 10.7±7.8%, respectively, which did not differ statistically from those in the group of intact animals (Fig. 4A, B, D).

The results of the study of the area under the platelet aggregation curve when inducing by ADP, collagen, and arachidonic acid against the background of the introduction of a selective arginase II inhibitor KUD 975 at a dose of 1 mg were 49.0±6.35%, 59.8±3.99%, 47.0±6.07%, respectively, and with the introduction of KUD 975 at a dose of 3 mg – 22.8±2.62%, 35.2±3.89%, 25.6±2.99%, respectively, which indicates a more pronounced pharmacological effect when the agent under study is administered at a higher dose.

The study of the effect of the combined administration of acetylsalicylic acid and a selective arginase II inhibitor KUD 975 on the area under the platelet aggregation curve when inducing by ADP, collagen, and arachidonic acid also indicates a significant decrease in the studied indicator by 13.9±5.9%, 6.0±4.1%, 11.0±6.0%, respectively (Table 1).
The obtained data convincingly indicate an increase in the antiaggregational effect of acetylsalicylic acid when used together with a selective arginase II inhibitor KUD 975. When studying plasma coagulation hemostasis, there is an improvement in the indicators of TT, PT, APTT, and fibrinogen against the background of the administration of acetylsalicylic acid at a dose of 7 mg and 10 mg, and KUD 975 at a dose of 1 mg and 3 mg. However, when using the studied drugs as monotherapy, the hemostasis indicators did not reach the target level. The combined use of acetylsalicylic acid at a dose of 10 mg and KUD 975 at a dose of 3 mg resulted in an increase in corrective effects on the links of plasma coagulation hemostasis. In the group of animals with combined administration of the studied pharmacological agents, the time indicators slightly exceeded the corresponding indicators in the group of animals with physiologically proceeding pregnancy. When assessing the levels of APPT, PT, and fibrinogen in the group of animals with combined administration of acetylsalicylic acid at a dose of 10 mg and KUD 975 at a dose of 3 mg, the results comparable to those of intact animals were obtained (Table 2).

Simulation of ADMA-like preeclampsia led to an increase in platelet aggregation ability when using all the aggregation inducers. This is evidenced by an increase in a degree, rate of aggregation, and a shortened time...
of thrombus formation. The shift of hemostasis towards proaggregation is explained by eNOS blockade, which leads to deficiency of NO in the endothelium and platelets.

The use of acetylsalicylic acid led to the correction of emerging disorders. The degree of aggregation and its rate increased to a lesser extent, and the time of thrombus formation was reduced to a lesser extent. The mechanism of action of acetylsalicylic acid involved blocking cyclooxygenase with a decrease in the formation of thromboxane (Dzeshka et al. 2016) and the ability to acetylate lysine eNOS. This leads to its activation and increased synthesis of NO not only in endothelial cells, but also in platelets (Estevez and Du 2017). Partial prevention of an increase in platelet aggregation capacity when using different aggregation inducers indicates the involvement of a NO-dependent mechanism.

When using a selective arginase II inhibitor, KUD 975, there was also a partial prevention of a shift of the hemostasis system towards proaggregation. The degree of aggregation and its rate increased to a lesser extent, and the time of thrombus formation was reduced to a lesser extent. The mechanism of action of a selective arginase II inhibitor KUD 975 is the inhibition of arginase II. At the same time, the bioavailability of L-arginine increases. This leads to substrate activation of eNOS and an increase in the substrate for the formation of NO (Gureev et al. 2015; Netrebko et al. 2021). Partial prevention of an increase in the aggregation capacity of platelets also occurs when using different aggregation inducers.

When using a combination of acetylsalicylic acid and a selective arginase II inhibitor KUD 975, the effect is enhanced. This fact is explained by the increase in the application points of the anti-aggregation effect of the drugs.

## Conclusion

The data obtained convincingly indicate a promising outlook for using acetylsalicylic acid and a selective arginase inhibitor KUD 975 in order to correct morpho-functional disorders in preeclampsia.

## Conflict of interests

The authors declare no conflict of interests.

### References

Gureeva AV et al.: Study of the effect of acetylsalicylic acid and a selective arginase...


Author contributors

- Anastasia V. Gureeva, 3-year student of the Faculty of Medicine; e-mail: nastasyi.207@gmail.com. The author took part in planning the experiments, analyzed the literature and participated in interpreting the data.

- Olga V. Severinova, postgraduate student of Department of Pharmacology and Clinical Pharmacology; e-mail: frendic@mail.ru, ORCID ID https://orcid.org/0000-0003-3873-0773. The author had a leading role in planning and performing the experiment, analyzing the data and literature and writing the article.

- Vladimir V. Gureev, Doctor Habil. of Medical Sciences, Associate Professor, Professor of the Department of Pharmacology and Clinical Pharmacology; e-mail: produmen@mail.ru, ORCID ID https://orcid.org/0000-0003-1433-1225. The author took part in planning the experiment, analyzed the literature and participated in interpreting the data.

- Indira S. Kochkarova, postgraduate student, Research Institute of Pharmacology of Living Systems; e-mail: kokhkarova@bsu.edu.ru. The author was engaged in collection, analysis and interpretation of the data for the paper.

- Elena V. Avdeyeva, Doctor Habil. of Biological Sciences, Professor of the Department of Normal Physiology; e-mail: avdeyeva.ev@mail.ru, ORCID ID http://orcid.org/0000-0002-7152-5483. The author consulted on planning, methodology and implementation of the experiment.