UDC 606:61 **https://doi.org/10.15407/biotech16.02.037**

Ex vivo STUDY OF THE ACTION OF INTEGRIN RECEPTORS ANTAGONIST FROM ECHIS MULTISQUAMATIS SNAKE VENOM ON PLATELETS OF PREGNANT WOMEN WITH COMPICATIONS DURING GESTATION

O. M. PLATONOV[1], *I. V. US*[2]

[1]Palladin Institute of Biochemistry
of the National Academy of Sciences of Ukraine, Kyiv
[2]The Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine

E-mail: chaosplaton@gmail.com

Received 2023/03/23
Revised 2023/04/05
Accepted 2023/04/28

Disorders of hemostasis system during gestation period is worldwide problem that influence, more or less, life of approximately 1/8 pregnant women [1]. The application of different methods to study problems that occur during pregnancy will allow defining and solving the risks during this period and give solutions for the preventing of complications. Elevated platelet function is an important part of pathogenesis of placenta dysfunctions and also a risk-factor for thrombotic events during pregnancy.

The issue of resistance to the most important antiplatelet drugs remains quite relevant. Aspirin is the most important antiplatelet agent in obstetrics, which has been proven as a means of preventing vascular-placental complications. At the same time, aspirin, as the gold standard of antiplatelet strategy, does not show the expected antiplatelet effect in 5–40% of patients [2]. Therefore, the search for new effective antiplatelet drugs and controlled antithrombotic prophylaxis to prevent placenta-associated pregnancy complications is an important task.

Aim. In our work, we studied platelet aggregation in blood plasma of pregnant women and estimated the possibility of *ex vivo* normalization of aggregation rate using a polypeptide from the *Echis multisquamatus* snake venom. Previous reports demonstrated that it directly interacts with glycoprotein IIb/IIIa receptors on the surface of platelets, preventing their adhesion, thereby affecting the degree of aggregation [3].

Methods. Crude venom of *Echis multisquamatus* was fractionated using ion-exchange chromatography followed by size-exclusion chromatography on Superdex 75 using the FPLC system (ÄKTA, GE Healthcare, USA). Analysis of molecular weight of protein components was performed using SDS-PAGE. The concentration of protein was measured using spectrophotometer Optizen POP (Korea) at 280 nm. The ability of obtained protein to inhibit platelet aggregation was measured directly by aggregometry. Blood samples of women with placental disfunction during pregnancy (n = 28) were kindly provided by "Perinatal Center of Kyiv". This study was approved by the Ethics Commission of the Shupyk National Medical Academy of Postgraduate Education and the Ethics Commission of the Kyiv Perinatal Center (# 3 from 05/05/2020). Aggregation of platelet-rich plasma (PRP) induced by ADP was investigated using aggregometry on the AP 2110 (Solar, Belarus). We compared the rate of platelet aggregation in the presence vs absence of platelet aggregation inhibitor.

Results. Two-step chromatography protocol allowed us to obtain the polypeptide from the venom of *Echis multisquamatus* that possessed the anti-aggregatory action. SDS-PAGE analysis confirmed the homogeneity of obtained polypeptide with apparent molecular weight 14 kDa that corresponds to the platelet aggregation inhibitor reported earlier [3]. Initial studies of ADP-induced platelet aggregation allowed selecting active concentration for the effective inhibitory action as 0.02 mg/ml.

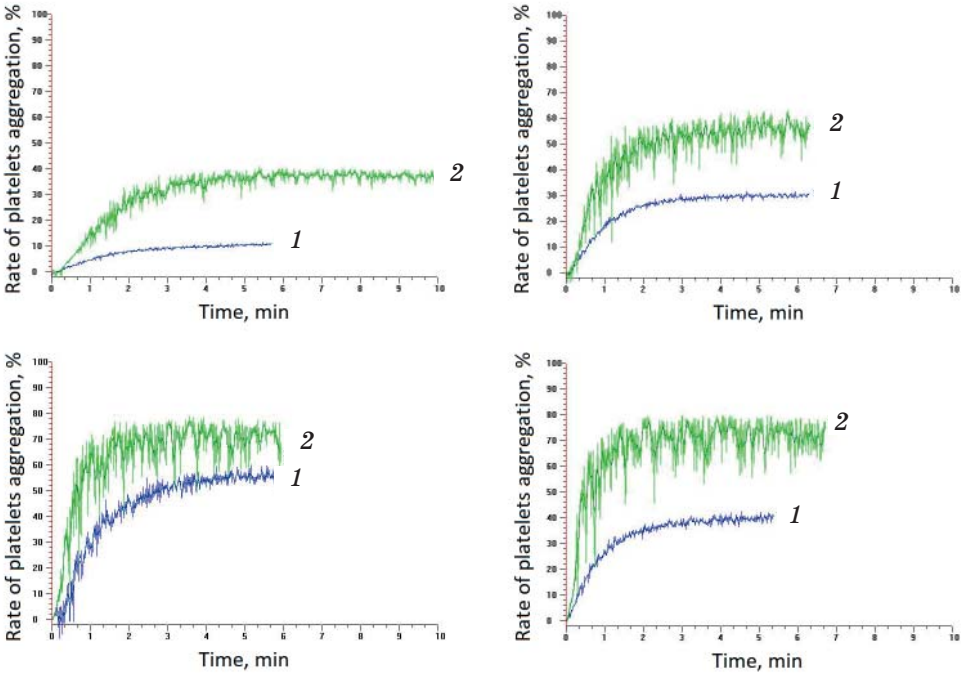

Figure. **ADP-induced aggregation of platelets in the platelet rich blood plasma of pregnant women with complicated pregnancy:**
1 — with adding of integrin receptors antagonist, *2* — control sample

Platelet aggregation in platelet rich plasma of pregnant women was measured immediately after blood collection. Polypeptide was added to the PRP at the final concentration 0.02 mg/ml. Platelets were activated by ADP immediately after that without pre-incubation. Inhibitory effect of studied polypeptide was shown for all analyzed samples of platelet rich blood plasma without the exceptions (Figure). In particular, it inhibited ADP-induced platelet aggregation in the range of 30–60%.

Discussion. Integrin receptors antagonists bind to receptors mainly through tripeptide motifs RGD [4]. Drugs based on RGD-containing snake venom disintegrins effectively inhibit platelet aggregation by blocking binding between neighboring platelets through its main ligand fibrinogen [5]. Effective inhibitory effect on platelets aggregation provides new opportunities for the use of disintegrins to develop the universal anti-aggregatory approaches that can be effective independently of individual peculiarities of patients. Taking into account the fact that integrins function not only as intercellular adhesive receptors, but also as cell-substrate receptors and are of great importance in the migration of leukocytes to the center of inflammation, further studies of anti-integrin agents in patients with vascular-placental complications, when the processes of sterile inflammation in the placenta are activated [6], look quite attractive.

Conclusions. Platelet aggregation inhibitor from *Echis multisquamatis* snake venom of can be assumed as the effective agent that reduce the rate of platelet aggregation. We demonstrated it efficacy in platelet rich plasma of pregnant women that had placenta dysfunction. The use of direct antagonist of platelet integrin receptors was assumed as the prospective approach for suppressing of platelet reactivity in particular during complicated pregnancy.

Key words: snake venom; disintegrin; aggregation of platelets; pregnancy; glycoprotein IIb/IIIa.

Ethical Committee Approval: # 3 from 2020/05/05/.

Author's contribution. O. M. Platonov performed ion-exchange chromatography, SDS-PAGE and aggregometry, I. V. Us supervised and monitored the patients.

Funding source. The research was financed by the project 0119U002512: "The interaction of the hemostasis system components at the cellular and molecular level in the process of formation and elimination of a thrombus.

Acknowledgement. Authors gratefully acknowledge Prof. S.I. Zhuk and Dr. V. O. Chernysheko for their contribution to this work.

REFERENCES

1. *Danilack V. A., Nunes A. P., Phipps M. G.* Unexpected complications of low-risk pregnancies in the United States. *American journal of obstetrics and gynecology*. 2015, 212(6), 809.e1–809.e6. https://doi.org/10.1016/j.ajog.2015.03.038
2. *Bij de Weg, J. M., Abheiden, C. N. H., Fuijkschot, W. W., Harmsze, A. M., de Boer, M. A., Thijs, A., & de Vries, J. I. P.* Resistance of aspirin during and after pregnancy: A longitudinal cohort study. *Pregnancy hypertension*, 2020, v. 19, p. 25–30. https://doi.org/10.1016/j.preghy.2019.11.008
3. *Chernyshenko V., Petruk N., Korolova D., Kasatkina L., Gornytska O., Platonova T., Chernyshenko T., Rebriev A., Dzhus O., Garmanchuk L., Lugovskoy E.* Antiplatelet and anti-proliferative action of disintegrin from Echis multisquamatis snake venom. *Croatian medical journal*. 2017, 58(2), 118–127. https://doi.org/10.3325/cmj.2017.58.118
4. *Vasconcelos A. A., Estrada J. C., David V., Wermelinger L. S., Almeida F. C. L., Zingali R. B.* Structure-Function Relationship of the Disintegrin Family: Sequence Signature and Integrin Interaction. *Frontiers in molecular biosciences*. 2021, v. 8, p. 783301. https://doi.org/10.3389/fmolb.2021.783301
5. *Lazarovici P., Marcinkiewicz C., Lelkes P. I.* From Snake Venom's Disintegrins and C-Type Lectins to Anti-Platelet Drugs. *Toxins*, 2019, 11(5), 303. https://doi.org/10.3390/toxins1105030
6. *Moser G., Guettler J., Forstner D., Gauster M.* Maternal Platelets-Friend or Foe of the Human Placenta?. *International journal of molecular sciences*, 2019, 20(22), 5639. https://doi.org/10.3390/ijms20225639