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Research Influence Biological Active Agents in the Course of Regulation of Functional Activity of Platelets and System of a Haemostasis

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Abstract

It is shown that the flavonoid pulikarin suppresses activity of an adenylate cyclase and reduces level intracellular [Ca^{2+}], perhaps its effect is connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages. Perhaps, oppression of fluorescence of membrane-bound Ca^{2+} is connected with inhibition of a pulikarin of release of calcium from intracellular depots. The inhibiting effect of a pulikarin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

Keywords: platelet, aggregation, forskolin, verapamil, pulikarin.

Introduction

Now the intracellular alarm system is the most popular model both for studying of the mechanism of action of biological active agent, and for screening of new medicines. It allows estimating influence of the studied substances on membrane receptors, Ca^{2+}-and Na^{+} exchange, and also the enzymes participating in synthesis and destruction of secondary intermediaries [1].

Definition of time of bleeding belongs to screening tests of studying of function of platelets. For the first time, this method was described to Dyuk in 1910, then improved by Ivy in 1941. The method can be regarded as the most ancient way of research of function of platelets and consists in definition of time from the moment of drawing a standard wound for skin before the termination of an effluence of blood. The analysis allows suspecting thrombocytopathiae of various

genesis, Ville brand's illness and violations the proagregant of properties of a vascular wall. Lack of lengthening of time of bleeding not always allows excluding hemostasia pathology (at violations of average degree of expressiveness less) [2].

One of the objects on which these researches are conducted, is platelets. These cages represent the excitable population of blood corpuscle, responsible for processes of coagulation, reparation of a vascular wall, deposition and transport of biological active agent, implementation of immune reactions of an organism [3].

Platelets represent highly specialized nuclear-free blood cells participating in many processes proceeding in an organism: in regeneration of the damaged fabrics, development of inflammatory, immune and allergic reactions. However their main function providing primary haemostasis, the important protective reaction preventing big blood loss at damage of vessels.

The blood platelets haemostasis is carried out by means of adhesion, swelling and formation of shoots of platelets, their aggregation and secretion, a retraction of a clot, a spasm of small vessels and formation of white blood platelets blood clot in microcirculation vessels. Participation of platelets in a haemostasis is defined by also angio trophic function and procoagulant properties (in the course of activation negatively loaded phospholipids move on an external membrane of platelets and are involved in the cascade of folding of plasma factors).

The methods of research existing today allow studying almost each stage of participation of platelets in the course of a blood formation.

Research of functional activity of platelets actually for definition of the reasons of different types of bleeding and thrombosis, implementation of selection of specific methods of prevention and treatment, the prevention of postoperative bleedings and thromboembolic; solutions of problems of habitual not incubation of pregnancy at an anti-phospholipid syndrome, the thrombophilia, the coagulopathy; control of efficiency and safety of therapy by anti-modular preparations. The developed research of aggregation functions of platelets is conducted for an assessment of safety of platelets when performing plasmas and a citeferez, hemosorption, use of cardiopulmonary bypasses and a haemodialysis, at storage the platelet of transfusion environments [4-7].

At activation from platelets over ten chemicals - aggregation inductors are released. One of them comes out of storage granules, others are synthesized at activation. Search and the characteristic of the new connections which are selectively contacting with receptors of a plasmatic membrane of platelets will give the chance of pharmacological regulation of functional activity of platelets [8].

Materials and methods

Animals and Ethics statement: This study was carried out in the Laboratory Electrophysiology of Institute of Bioorganic Chemistry of Academy Sciences of the Republic of Uzbekistan on physically fit, adult, albino rats in both sexes (female and male) obtained from the vivarium of the Laboratory of Pharmacology. Animals had been fed with standard food and water in the vivarium. In all experiments albino rats weighing 200 – 250 g were used (n = 18). During the experiments, while working with experimental animals, International principles of the Helsinki Declaration and the rules of human attitudes towards animals were completely followed.

Solvents and chemicals: Platelets allocated with a centrifugation method at 1500 rpm, within 15 min., for sedimentation of erythrocytes. The plasma enriched with platelets was centrifuged repeatedly within 10 min. at 3 thousand rpm. A deposit of platelets of a suspended in 5 ml of the environment containing *150 mm of NaCl, 2,7 mm of KCl, 0,37 mm of NaH_2PO_4, 1 mm of $MgCl_2$, 1 mm of $CaCl_2$, 5 mm glucose, 10 mm of HEPES-NaOH, pH 6,55, 50 of piece/ml of heparin, 0,35% of serumal albumine and 0,15 mg/ml of an apyrase*. Aggregation of platelets was registered on Born's method [9]. As inductors of aggregation of platelets used ADP (2 microns), adrenaline (5 microns) and thrombin (0,5 pieces/ml) (Sigma).

For an assessment of influence of the studied connections on the level of intracellular calcium used a fluorescent method of registration according to Tsien [10].

Data analysis. The statistical importance of distinctions between controlled and skilled values was defined for a number of data, using the pair t-test where controlled and skilled values are taken together, and the unpaired t-test if they are taken separately. Value P<0, 05 indicated

statistically significant distinctions. The received results are statistically processed on Origin 6.1 (Origin Lab Corporation, the USA).

Results and discussion

At Institute of the Chemistry of Plant Substances, Academy of Sciences Republic of Uzbekistan, researches on studying of pharmacological properties of vegetable substance of a pulikarin were conducted. Pulikarin is one of perspective connections possessing potential tire-tread and anti-toxic action. However at what level their action is realized and what their molecular mechanisms remain not studied. In this regard, in experiments action of a flavonoid of a pulikarin on system of a haemostasis of a blood of rats was investigated. In the real work influence of a flavonoid of the pulikarin (allocated from plants of *P.salviifolia* which represents *6,3 '-digidroksi-3,5,7,4 '-tetrametoksiflavon*) on system of a fibrillation was studied.

Pulikarin in concentration of 60 microns in plasma rich and poor in platelets didn't cause fibrillation of plasma in vitro. But at research of influence of a pulikarin on thrombin and thrombin is more similar effects of poisons of snakes (*Vipera lebetina, Echis multisquamatus and Akqistrodon halys*) is revealed that pulikarin more dose dependent reduces influence of thrombin and these poisons (0,01g/ml) on process of a blood clot formation and of a fibrinous clot in the plasma rich with platelets. If to consider that antitromb action of a pulikarin is shown more in plasma rich with platelets, perhaps, its action is connected with secretion inhibition from platelets of activators of a fibrillation (a thromboxane of A_2, ions of Ca^{2+}, the factor of activation of platelets (FAP), fibrinogen and many others).

In preliminary researches influence of the pulikarin on functional activity of platelets isn't revealed, however pulikarin dose dependent inhibited thrombin, adrenaline and ADP-induced aggregation of platelets. Thus the most inhibiting property of a pulikarin was observed at ADP-induced aggregations (fig. 1.) pulikarin in concentration 50mkm caused 50% suppression ADP-induced of aggregation of platelets. Further increase of concentration of a flavonoid of a pulikarin to 80mkm and 100mkm led to almost full inhibition of aggregation of platelets.

Having contacted with specific receptors on a membrane of a platelet, ADP creates favorable conditions for reception of fibrinogen on a surface of platelets that the glycoprotein of receptors of IIb/IIIa leads to activation [11]. ADF is the natural inductor of aggregation of platelets in the blood course, collects in dense granules of platelets and is allocated in the course of primary haemostasis. Working through purinoreceptor, ADF activates platelets owing to sharp increase level the intracellular calcium coming to cytosol as from extracellular space as a result of stimulation of activity of a phospholipase of (PLC), and also from intracellular depots, owing to start of a phosphoinositol way. Stimulation of Ca-dependent of enzymes leads to change of a form of platelets, the subsequent aggregation and secretion of biologically active agents from granules in extracellular space [12].

Adrenaline activates process of aggregation of platelets, causing stimulation α2– adrenergic receptors. Thus there is an oppression of an adenylate cyclase, reduction of the sAMF level and change of the maintenance of intracellular Ca^{2+}. Also adrenaline promotes activation of other agonist therefore, can lead to intra vascular aggregation of platelets [13].

It is known that ADP leads to sharp increase in intracellular concentration [Ca^{2+}], and this increase is carried out as due to activation of an adenylate cyclase, and release from intracellular storages.

To check whether it is connected the inhibiting action of a pulikarin of aggregation of platelets with oppression of activation of an adenylate cyclase, and release from intracellular storages, its action a verapamil background (a blocker of calcic channels) and a forskolin (the adenylate cyclase activator) is investigated.

Thus it is revealed that against a background verapamil and a forskolin in the concentration, for 50% reducing ADP-induced aggregation of platelets, the inhibiting effect of a flavonoid of a pulikarin amplified.

The received results show that the inhibiting effect of a pulikarin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

With the purpose of specification of some mechanisms of antiagregant action of a flavonoid of a pulikarin, its influence on the level of intracellular and membrane-bound Ca^{2+} with use of

fluorescent probes Fura-2/AM and chlortetracycline (CTC) was investigated. To define, whether action of a pulikarin on a gain of cytoplasmatic concentration of Ca^{2+} is based, the induced ADP, experiment was made at presence and without physiological concentration of Ca^{2+}.

In control at presence and without physiological concentration of Ca^{2+} the fluorescence gain Fura-2/AM and CTC induced by ADP is revealed.

At research of action of a pulikarin on fluorescence gain Fura-2/AM induced by ADP for lack of extracellular Ca^{2+} it is revealed that pulikarin dose dependent release of Ca^{2+} from intracellular depots oppresses. Thus full suppression of a gain of cytoplasmatic concentration of Ca^{2+} wasn't observed (fig. 1).

At the same time against a background a pulikarin, in the presence of extracellular Ca^{2+} the fluorescence Fura-2/AM induced by ADP was much more, than in lack of extracellular Ca^{2+} that says that pulikarin oppresses only release of Ca^{2+} from intracellular depots (fig. 1).

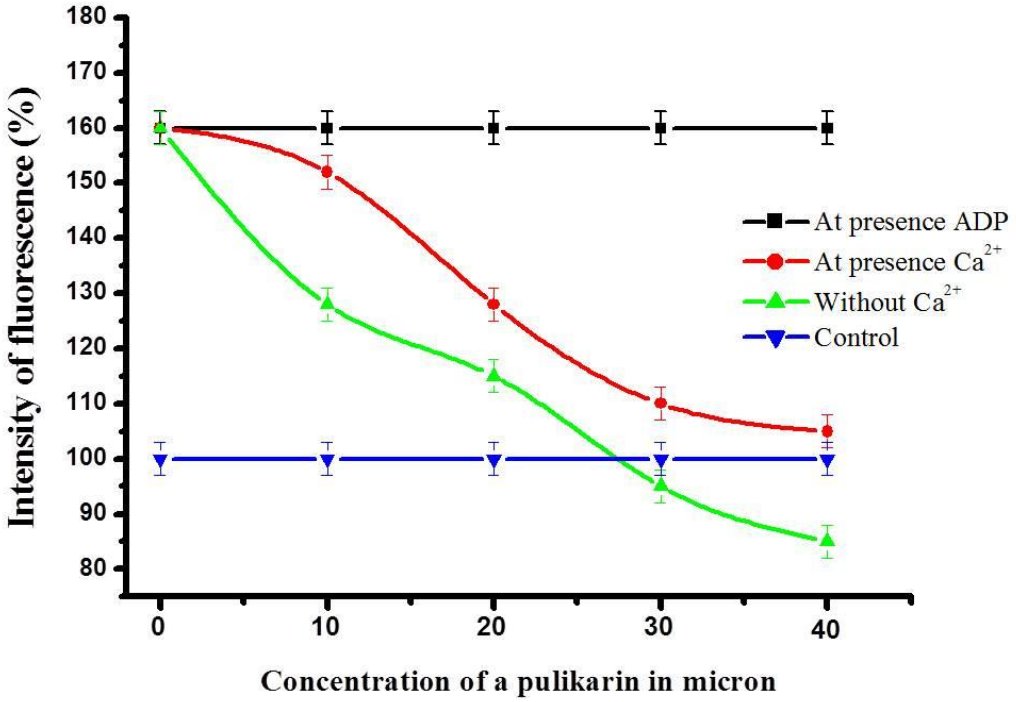

Fig. 1. A fluorescence gain at ADP-induced aggregations under the influence of a pulikarin. Reliability indicator: P<0,05

These assumptions are confirmed in researches of action of a pulikarin against a background blocker of Ca^{2+} verapamil. Against a background verapamil pulikarin the gain of level of intracellular Ca^{2+}, the induced ADP (fig. 2) slightly oppressed.

At linkng of ADP with the corresponding receptors on a membrane of platelets, intermediate connections which stimulate release of calcium from depot are formed.

In researches of action of a pulikarin against a background a forskolin, it is revealed that pulikarin dose dependent strengthened the inhibiting action of a forskolin on ADP-induced increase of intracellular calcium (fig. 2). These results show that the flavonoid pulikarin suppresses activity of an adenylate cyclase and reduces level intracellular [Ca^{2+}], perhaps its effect is connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages.

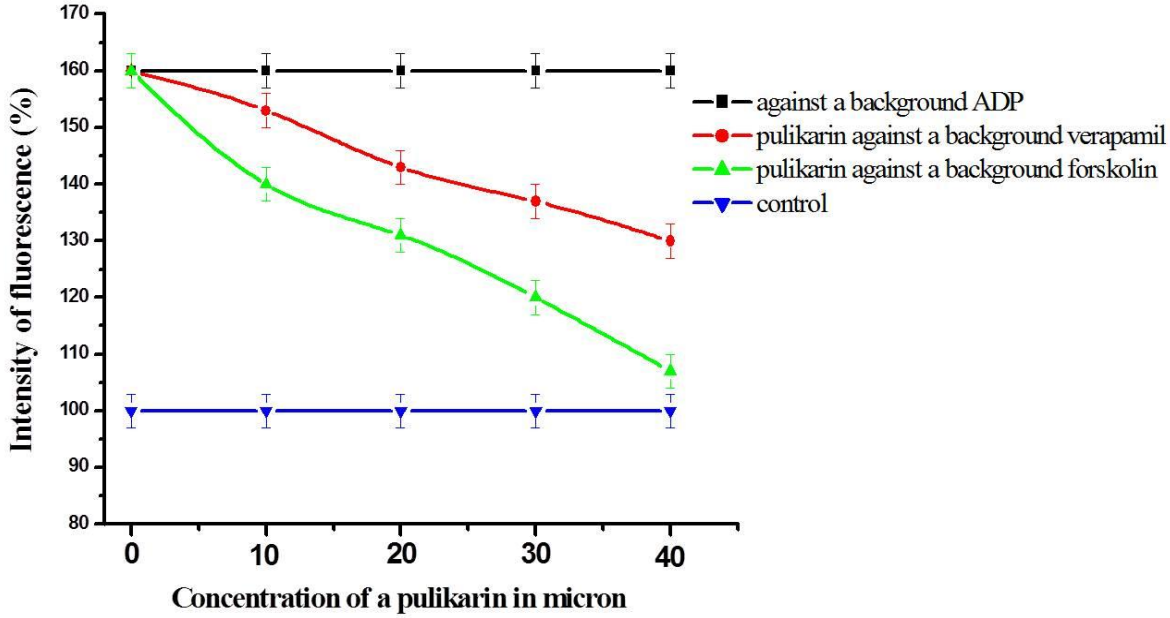

Fig. 2. A fluorescence gain at ADP-induced aggregations under the influence of a pulikarin, against a background blockers of verapamil and a forskolin. Reliability indicator: P<0,05

In a case with use of fluorescent probes of CTC, against a background a pulikarin considerable oppression of fluorescence of membrane-bound Ca^{2+} in lack of physiological concentration of Ca^{2+} was also observed.

Conclusion

Received results show that flavonoid pulikarin suppresses activity of an adenylate cyclase and reduces level intracellular [Ca^{2+}], perhaps its effect is connected with inhibition of a gain of cytoplasmatic Ca^{2+} as at the expense of its entrance outside, and release from intracellular storages. Perhaps, oppression of fluorescence of membrane-bound Ca^{2+} is connected with inhibition of a pulikarin of release of calcium from intracellular depots. The received results show that the inhibiting effect of a pulikarin on ADP-induced aggregation of platelets is connected with oppression of a gain of cytoplasmatic concentration of Ca^{2+} from depot of platelets.

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Исследование влияние биологические активные вещества в процессе регуляции функциональной активности тромбоцитов и систему гемостаза

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Аннотация. Показано, что флавоноид пуликарин подавляет активность аденилатциклазы и снижает уровень внутриклеточного [Ca^{2+}], возможно его эффект связан с ингибированием прироста цитоплазматического Ca^{2+} как за счет его входа снаружи, так и высвобождения из внутриклеточных хранилищ. Возможно, угнетение флуоресценции мембраносвязанного Ca^{2+}, связано с ингибированием пуликарина высвобождения кальция из внутриклеточных депо. Ингибирующий эффект пуликарина на АДФ-индуцированную агрегацию тромбоцитов связан с угнетением прироста цитоплазматической концентрации Ca^{2+} из депо тромбоцитов.

Ключевые слова: тромбоцит, агрегация, форсколин, верапамил, пуликарин.