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CHANGE IN THE HEMOSTASIS SYSTEM UNDER THE INFLUENCE OF AN HSS-23

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ABSTRACT: In this work, the modified polysaccharide GSS-23 was studied for the functional activity of platelets. It has been determined that inhibition of the level of fluorescence of membrane-bound Ca2+ under the influence of GSS-23 is possibly associated with inhibition of calcium release from the depot. It has been shown that the inhibitory effect of GSS-23 on ADP-

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induced aggregation is associated with inhibition of the increase in the cytoplasmic concentration of Ca2+ from the platelet depot.

KEYWORDS: Intracellular Ca2+, cytosolic Ca2+, platelets, ADP, EGTA.

INTRODUCTION

Anticoagulants have a pronounced effect on all phases of blood coagulation, therefore the study of their activity in coagulation disorders is very important. In this regard, sulfated polysaccharides are unique compounds acting on individual units of the hemostasis system. Platelets are small, nuclear-free structures formed from megakaryocytes in the bone marrow. The functional role of platelets in the human body is very diverse. Thus, platelets are involved in the protection of the host organism from viruses and bacteria, transport of substances, regulation of vascular tone, growth, metastasis, and destruction of cancer cells, in angiogenesis and vascular remodeling, etc. The main function of platelets is to participate in blood coagulation (hemostasis) - a process that prevents blood loss when a blood vessel is damaged. In the course of many years of studies of platelet functions, it was shown that they play an essential role in such important processes for the body as primary hemostasis, regulation of vascular tone, inflammation, exercise a protective effect, and others [1]. Thrombosis is an intravital blockage of a blood vessel (artery or vein) with coagulated blood. Among the many reasons contributing to the occurrence of vascular thrombosis, an increase in the coagulating properties of blood is of great importance. Thrombosis is the result of an increased formation of thrombin as an excessive reaction of the hemostasis system to damage to the vascular wall or any other pathological process in the body [2]. The mechanism of action of alkaloids is different from that of heparin. Alkaloids directly inhibit thrombin without the involvement of plasma serine proteinase inhibitors compared to heparin. No direct inhibition of factor Xa was found [3]. Alkaloids inhibit the internal coagulation pathway at low doses, and at high doses, the external coagulation pathway. It was revealed that alkaloids do not possess acute toxicity, but have a broad therapeutic effect.

The aim of this work was the effect of the modified polysaccharide HSS-23 obtained from plant materials (pectin, alginic acid) on the functional activity of platelets.

Material and methods: outbred white rats weighing 200 g, contained on the vivarium ration, 5-6 rats per cage, at a humidity of 70-80%, a temperature of 24-26°C, with free access to water and food, were used in the work. To study the coagulation activity of the alkaloid, we used citrate blood plasma taken from the tail vein of rats, prepared on sodium citrate 3.8%, in a ratio of 1: 9. Platelets were isolated by centrifugation at 1150 rpm for 15 minutes to precipitate erythrocytes. The platelet-rich plasma was re-centrifuged for 10 min. at 3 thousand rpm The platelet pellet was suspended in 5 ml of medium containing 150 mM NaCl, 2.7 mM KCl, 0.37 mM NaH_2PO_4, 1 mM $MgCl_2$, 1 mm $CaCl_2$, 5 mM glucose, 10 mM HEPES-NaOH, pH 6.55, 50 unit's/ml heparin, 0.35% serum albumin and 0.15 mg/ml apyrase [4]. All operations were performed in plastic containers at room temperature.

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Measurement of intracellular Ca2+: To measure the amount of membrane bound Ca2+, 20 μM chlortetracycline (CTC) was added to platelets placed in a medium similar to that used for cell isolation, but without apyrase and MgCl2. They were incubated for 60 min to achieve the maximum interaction of CTC with membrane-bound Ca2+, both on the plasma and intracellular membranes. The excitation wavelength of CTC is 405 nm; registration is 530 nm. The results were expressed as a percentage, taking as 100% the difference between the maximum value of the fluorescence intensity (fluorescence of the dye saturated with Ca2+) and its minimum value (fluorescence of the indicator in the absence of Ca2+) obtained after the addition of EGTA.

To measure free cytosolic Ca2+, platelets (1×108 cells/ml) were loaded with 4 μM Fura-2AM acetoxymethyl ether for 40 min at 37°C. At the same time, in the dye molecules penetrated into the cytoplasm, under the action of intracellular esterases, the ether group is cleaved as a result of the formed Fura-2 anion, binds Ca2+. Upon completion of the loading, the dye remaining in the medium was removed by double washing and centrifugation in the standard medium. In the experiments, the cell concentration in the cell was 5×106 cells/ml. Excitation of fluorescence was caused at 337 nm, and registration of fluorescence at 496 nm. The fluorescence of the dye (Fmax) saturated with Ca2+ was determined by adding 50 μM digitonin to the cells loaded with Fura-2AM. Fmin was determined by measuring the fluorescence intensity in a calcium-free medium, Fmin = [(Fmax - Faf)/3] +Faf, where Faf is the auto fluorescence of cells determined by the addition of 0.1 mM MnCl2 to platelets loaded with Fura-2AM and treated with digitonin [5].

RESULTS AND DISCUSSION

The effect of the HSS-23 on the level of intracellular and membrane-bound Ca2+ platelets were studied using fluorescent probes Fura-2/AM and CTC. It is known that adenosine diphosphate (ADP) leads to a sharp increase in the intracellular concentration of Ca2+. In order to determine whether the effect of HSS-23 is based on an increase in the cytoplasmic concentration of Ca2+ induced by ADP, the experiment was carried out in the presence and without physiological concentrations of Ca2+. In the control, in the presence and without physiological concentrations of Ca2+, an increase in the fluorescence of Fura-2/AM and CTC, induced by ADP, was revealed. When studying the actions of HSS-23 on the increase in Fura-2/AM fluorescence induced by ADP in the absence of extracellular Ca2+, it was found that HSS-23 s dose-dependently inhibits the release of Ca2+ from intracellular stores. At the same time, complete suppression of the increase in the cytoplasmic concentration of Ca2+ was not observed. At the same time, against the background of HSS-23, in the presence of extracellular Ca2+, the Fura-2/AM fluorescence induced by ADP was significantly higher than in the absence of extracellular Ca2+, possibly only the release of Ca2+ from intracellular stores inhibits HSS-23.

These assumptions are confirmed in studies of the action of HSS-23 against the background of verapamil. It was shown that HSS-23 s insignificantly inhibited the increase in the level of intracellular Ca2+ induced by ADP. When ADP binds to the corresponding receptors on the platelet membrane, intermediate compounds are formed that stimulate the release of calcium from the depot. In studies of the action of HSS-23 against the background of forskolin (adenylate cyclase activator), it was found that HSS-23 s dose-dependently increased the inhibitory effect of forskolin on ADP-induced increase in intracellular calcium. In the case of using CTC fluorescent

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probes, against the background of HSS-23, significant inhibition of membrane-bound Ca2+ fluorescence was also observed in the absence of physiological Ca2+ concentrations. Possibly, inhibition of membrane-bound Ca2+ fluorescence is associated with inhibition of calcium release from the depot. It was shown that the inhibitory effect of HSS-23 on ADP-induced aggregation is associated with inhibition of the increase in the cytoplasmic concentration of Ca2+ from the platelet depot.

Regulation of changes in the level of intracellular and membrane-bound Ca2+ platelets using HSS-23 gives in the future anticoagulant, fibrinolytic and antithrombotic drugs.

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