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INFLUENCE OF SOME SULPHATED POLYSACCHARIDES ON THE PLATELET AGGREGATION IN NORMAL AND IN PATIENTS WITH ISCHEMIC HEART DISEASE

[1]Nasirov K.E., [2]Musaeva M.K., [3]Khoshimov N.N., [4]Raimova G.M., [5]Turaev A.S., [6]Muhitdinov B.I.

ABSTRACT

Purpose of the research: *To study the effect of various modified sulfated polysaccharides on platelet aggregation activity in normal and in patients with coronary heart disease (CHD).* ***Materials and methods:*** *Platelet aggregation was recorded on a Biol ALAT-2 220LA aggregometer (Russia) on rich plasma of a healthy person and coronary heart disease. Platelet aggregation inducers used were ADP (2 μM), adrenaline (5 μM), and collagen and ristomycin (0.5 u / ml) (Sigma).* ***Results:*** *The degree of aggregation with the addition of ADP or adrenaline inducers in the blood plasma of a healthy person and coronary heart disease is shown, depending on. In contrast to ADP and adrenaline-induced aggregation, in collagen or ristomycin-induced aggregation, in the blood plasma of a healthy person and CHD, an almost identical single-phase, irreversible curve was observed, which shows that collagen and ristomycin-induced platelet aggregation do not change significantly. When studying the effect of compounds CC-BOS-122, CC-GSC-63, CC-GSC-14 on platelet aggregation in healthy human plasma and coronary heart disease, it was shown that the compound CC-BOS-122 has the most pronounced activity.* ***Conclusions****: The studied compounds CC-BOS-122, CC-GSC-63, and CC-GSC-14 have the greatest effect on the activity of glycoprotein receptors on the platelet membrane due to the mobilization of calcium ions from intracellular depots.*

Keywords: *coronary heart disease, platelet glycoprotein receptors, platelet aggregation, sulfated polysaccharides.*

I. Introduction

Cardiovascular disease (CVD) is a huge socio-economic problem in the structure of mortality and disability [1, 2, 4]. At present, the role of such risk factors for CVD as coronary heart disease (CHD), arterial hypertension (AH),

[1] Institute of Biophysics and Biochemistry at the National University of Uzbekistan
[2] Institute of Biophysics and Biochemistry at the National University of Uzbekistan
[3] Institute of Biophysics and Biochemistry at the National University of Uzbekistan
[4] Institute of Biophysics and Biochemistry at the National University of Uzbekistan
[5] Institute of Bioorganic Chemistry named after academician A.S. Sadykov Uzbek Academy of Sciences
[6] Institute of Bioorganic Chemistry named after academician A.S. Sadykov Uzbek Academy of Sciences

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atherosclerosis, cardiac arrhythmias, which underlies a key role in impaired platelet functional activity, has been studied quite well [5, 13, 14]. Platelets, acting on platelet hemostasis, cause the development of blood clots and the consequences of acute vascular complications [9, 14, 15, 16].

Reliable data have been obtained that the administration of antiplatelet drugs based on sulfated polysaccharides reduces the risk of developing acute vascular complication, including ischemic strokes [3, 8, 10]. Sulfated polysaccharides have a wide range of anticoagulant effects and are likely to affect platelet aggregation [10, 12]. For example, heparin, by inactivating thrombin, it can reduce or prevent platelet aggregation [6, 7]. On the other hand, heparin is able to enhance platelet aggregation caused by other inducers (in addition to thrombin), and this property of it to a certain extent depends on molecular weight. In this regard, research and analysis of new sulfated polysaccharides with different mechanisms of action is relevant for the search and creation of specific antiaggregate pharmacological preparations.

Goal of the research: to study the effect of some modified sulfated polysaccharides on platelet aggregation activity in normal and in patients with coronary heart disease.

II. Material and methods

Three sulfated polysaccharides, which differ in the presence or absence of a structure (Fig. 1), were used in the studies.

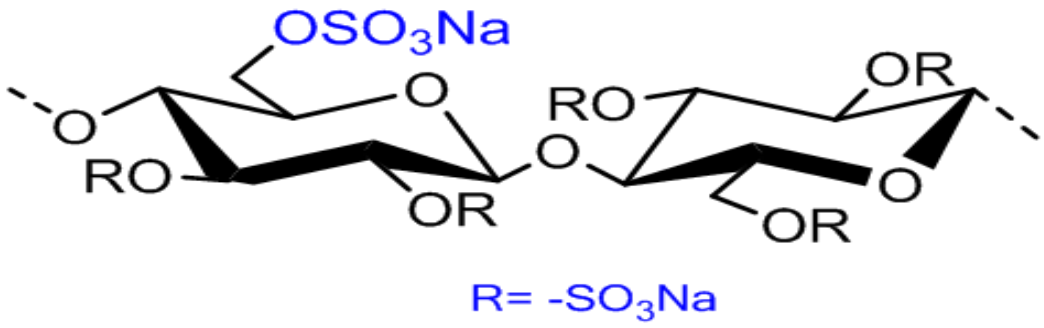

Fig. 1. General structural formulas of modified sulfated polysaccharides

To study platelet aggregation activity, normal and in patients, rich plasma of blood was used in 43 patients who had a history of CHD and who received antiplatelet drugs, and 25 patients who made up the control group. The experiments were carried out in vitro. Platelets were isolated by centrifugation at 1150 rpm for 5 minutes to precipitate red blood cells. Platelet rich plasma was centrifuged again for 10 minutes. at 3 thousand rpm Platelet sediment was suspended in 5 ml of medium containing 150 mMNaCl, 2.7 mMKCl, 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 u / ml heparin, 0.35% serum albumin and 0.15 mg / ml apyrase. All operations were carried out in plastic dishes at room temperature. The most effective samples of sulfated polysaccharides CC-BOS-122, SC-GSC-63, and CC-GSC-14 were used in the work.

Platelet aggregation was recorded by the Born method [6] on a Biol ALAT-2 220LA aggregometer (Russia). Platelet aggregation inducers used were ADP (2 μM), adrenaline (5 μM), and collagen and ristomycin (0.5 u / ml) (Sigma). The process of aggregation was visualized graphically. The degree of platelet aggregation was expressed as% of the maximum transmittance (T%, max). To measure the amount of membrane-bound Ca^{2+} to platelets, a cell concentration of 5 × 106 cells / ml was used. Excitation of fluorescence was caused at 337 nm, and registration of

fluorescence at 496 nm. Dye fluorescence (Fmax) saturated with Ca^{2+} was determined by adding to Fura-2AM-loaded cells. Fmin was determined by measuring the fluorescence intensity in a calcium-free medium. ADP (0.5 μg / ml) was used as a stimulator of calcium release from intracellular depots. The chelating ability of MSCs in relation to calcium ions was studied in intact platelets in comparison with EGTA. The measurements were carried out using a spectrometer (USB-2000. USA) and a Hitachi fluorimeter, Japan).

Statistical data processing and illustrations were performed using the computer program Origin 6.1 (Microsoft, USA).

III. Results

In our previous experiments, it was shown that the modified sulfated polysaccharides SC-BOS-122 SC-GSC-14 and SC-GSC-63 lengthen the blood coagulation time to different degrees in the APTT, APTT, and prothrombin time, like heparin. The following results were obtained in these studies: CC-BOS-122, unlike the compounds CC-GSC-14 and CC-GSC-63, prolongs the thrombin time by APTT, and at lower concentrations it leads to prolonged and dose-dependent hypocoagulation.

It was shown that CC-BOS-122, unlike GSC-14, CC-GSC-63, dose-dependently lengthens the prothrombin time. By lengthening the prothrombin time against the background of compound CC-BOS-122, one can judge the inhibition of the activation factors of the external coagulation mechanism, i.e., the inhibition of the activity of factors V, II and VII, the formation of prothrombinase, its effect on prothrombin and the subsequent formation of fibrin. A comparison of the obtained results of APTT with prothrombin and thrombin time proves the effect of compounds CC-GSC-14, CC-GSC-63 and CC-BOS-122 on factors VIII, IX, X, XI, XII of blood coagulation. As a result of the prolongation of APTT and PV against the background of normal thrombin time, one can judge the inhibition of factors II, V, X. Thus, the change in the level of APTT does not depend on the number of platelets.

Using platelet aggregation inducers, we studied the effects of SMEs on platelet hemostasis under normal and CHD conditions. In the experiments, the effect of SMEs on spontaneous and platelet aggregation induced agonists was evaluated. Although it is known how the inducers of ADP, adrenaline, collagen, and ristomycin affect platelet function, in studies when evaluating their effect on platelet aggregation, one should establish their reversible values depending on the apparatus and reagents.

In this regard, the effect of the above agonists on the functional activity of platelets in the blood plasma of a healthy person and CHD in vitro was studied. When studying the blood plasma of a healthy person, spontaneous platelet aggregation was not observed. The degree of aggregation with the addition of an ADP inducer in the blood plasma of a healthy person, depending on the concentration (1-5 μg / ml), showed primary single-phase and secondary aggregation in the form of a two-phase curve, at high concentrations (10 μg / ml), irreversible platelet aggregation (fig. 2 a). Another adrenaline inducer at concentrations of 1-10 μg / ml, as well as ADP, dose-dependently induced platelet aggregation. Adrenaline at a concentration of 1 μg / ml caused aggregation in the form of a two-phase curve, and at higher concentrations it caused irreversible aggregation of platelets, which corresponds to the literature data (Fig.2b).

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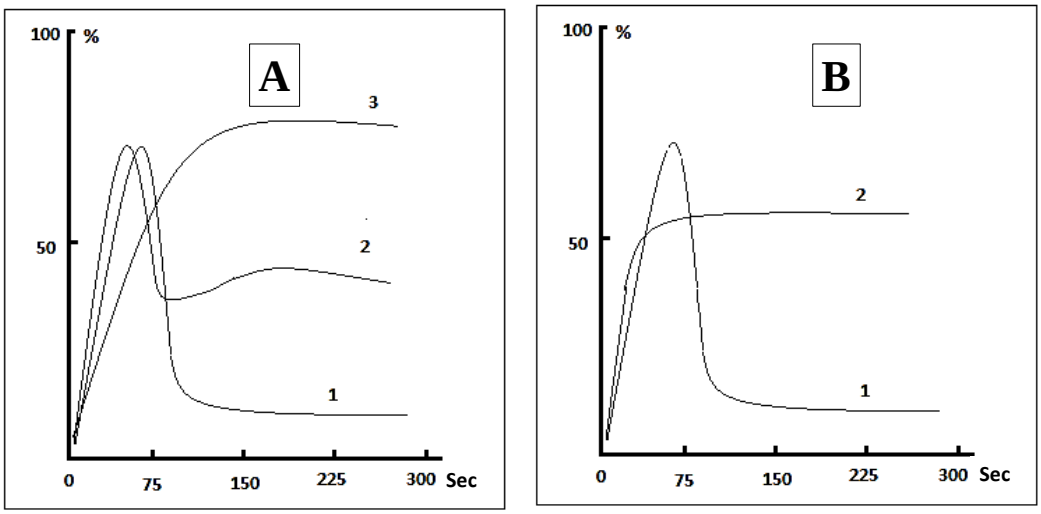

Fig. 2 (A) ADP-induced platelet aggregation of healthy human blood plasma: in concentrations 1) - 1 μg / ml; 2) - 5 μg / ml; and 3) 10 μg / ml.

(B) Adrenaline-induced platelet aggregation of healthy blood plasma: in concentrations of 1) - 1 μg / ml; 2) -5 μg / ml.

In the case of blood plasma of patients with coronary artery disease (platelet aggregation activity of the patient's blood plasma was evaluated upon admission of patients to the hospital), spontaneous platelet aggregation was observed. When indicated with ADP (1 μg / ml), both secondary aggregation was observed, so at concentrations of 5-10 μg / ml irreversible platelet aggregation was observed. When indicated with adrenaline, also, at low concentrations of 1-5 μg / ml, adrenaline caused irreversible platelet aggregation. As you know, the action of ADP is mediated through binding to the P2Y12 receptor, which plays a crucial role in platelet activation, including aggregation, secretion, and release of coagulation factors. Adrenaline displays a TXA2-dependent pathway for activation of blood platelets. The obtained results indicate activation of the P2Y12 receptor and the TXA2-dependent plasma platelet activation pathway in patients with coronary artery disease in comparison with the control.

In the next series, the effects of ADP, adrenaline, ristomycin, and collagen on platelet aggregation in a healthy person's blood plasma and coronary heart disease are studied (Fig. 3). As you know, ristomycin-induced platelet aggregation indirectly characterizes the activity of von Willebrand factor, while collagen-induced aggregation is the integrity of the endothelial layer.

In contrast to ADP and adrenaline-induced aggregation, in collagen and ristomycin-induced aggregation, in the blood plasma of a healthy person and in coronary heart disease, an almost identical single-phase, irreversible curve was observed, which indicates that collagen and ristomycin-induced platelet aggregation do not change significantly.

When studying the effect of MCP SC-BOS-122, SC-GSC-63, and SC-GSC-14 on platelet aggregation in healthy human plasma and coronary heart disease, a significant inhibitory effect of ICP was revealed when indicated with ADP and adrenaline. In cases with collagen, ristomycin-indication, the studied compounds did not have a noticeable effect on platelet aggregation, in some cases, on the contrary, they stimulated a little aggregation in the blood plasma of patients with coronary heart disease (Fig. 3.).

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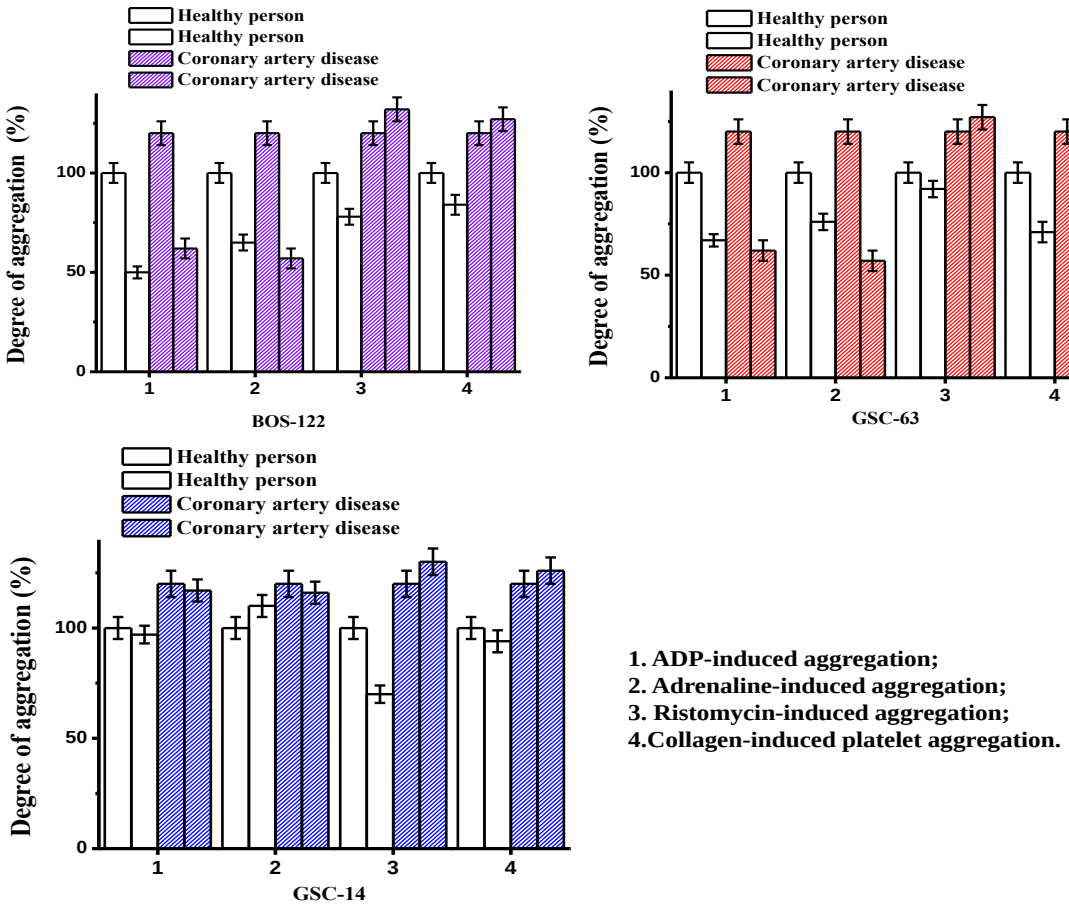

Fig. 3. The effect of CC-BOS-122, CC-GSC-63, CC-GSC-14 on platelet aggregation in healthy human plasma and CHD.

The results showed that the most pronounced inhibitory activity of SMEs is manifested in ADP-induced platelet aggregation, apparently due to inhibition of cyclooxygenase activity of platelets.

Moreover, the above polysaccharides had a dose-dependent nature of the action. Thus, the compound CC-BOS-122 at low concentrations of 1–2.5 μg / ml, induced platelet aggregation with an increase in max. values (10.54) and max. slope (9.025) of the aggregation curve (Fig. 4a). At higher concentrations, 10 μg / μl inhibited ADP-induced platelet aggregation by more than 50% and reduced max. value (8.09), max. slope (3.95) of the aggregation curve (Fig. 4a, b). Compound CC-GSC-14, like CC-BOS-122, at low concentrations caused platelet aggregation of CHD blood plasma with an increase in max. values (2.73) and max. the slope (3.04) of the aggregation curve (Fig. 4c). However, unlike the compound CC-BOS-122, the compound CC-GSC-14 at higher concentrations (10-25 μg / ml) did not cause inhibition of ADP-induced platelet aggregation (Fig. 4d).

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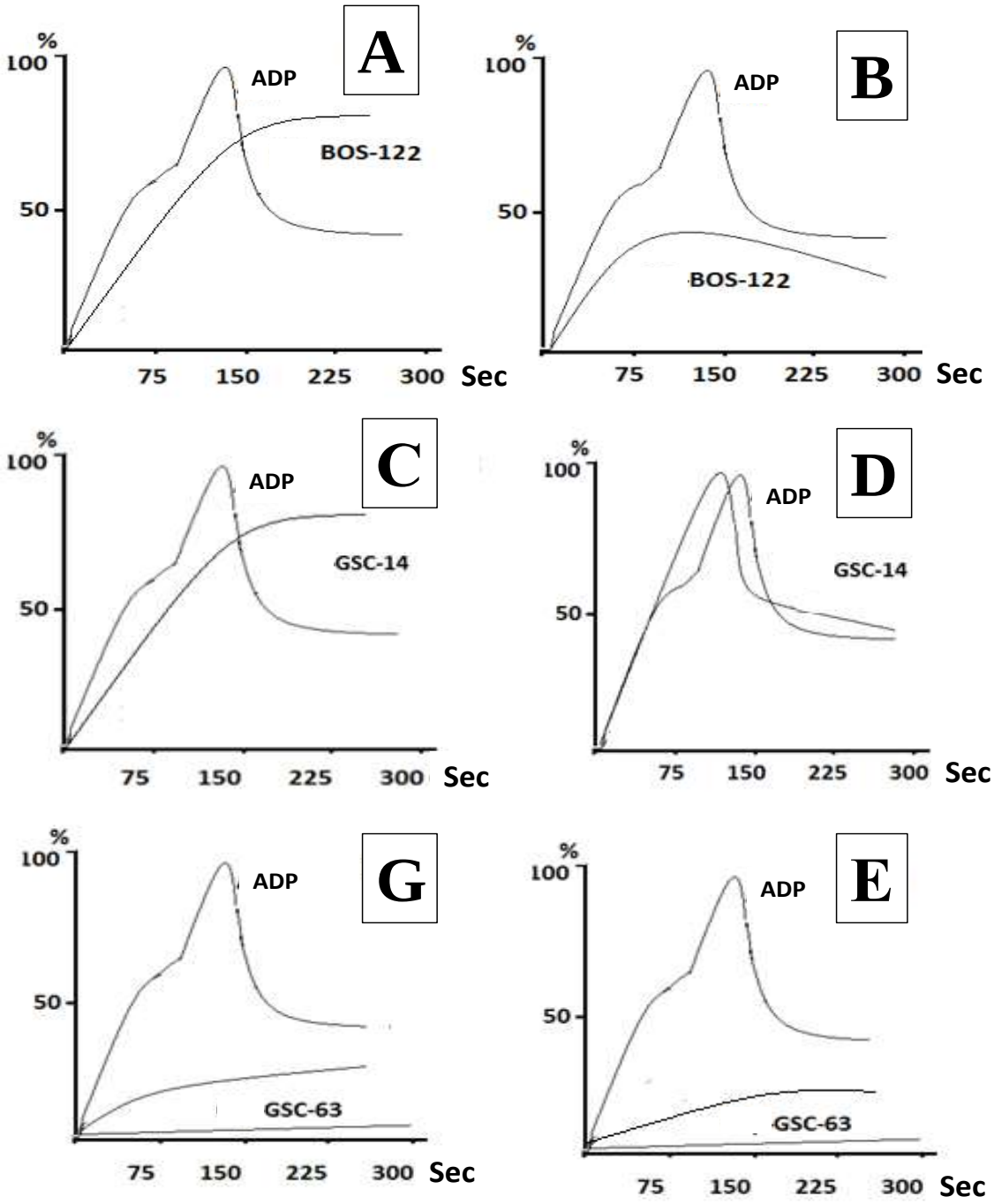

Fig. 4. The effect of CC-BOS-122 on ADP-induced platelet aggregation in ischemic heart disease, in concentrations A (1 μg / ml) and B (10 μg / ml). The effect of CC-GOS-14 on ADP-induced platelet aggregation in coronary heart disease in concentrations of C (1 μg / ml) and D (10 μg / ml). The effect of CC-GSC-63 on ADP-induced platelet aggregation in coronary heart disease at concentrations of G (1 μg / ml) and E (10 μg / ml).

The compound of sample CC-GSC-63, unlike the compound CC-GSC-14, CC-BOS-122 and at low concentrations, inhibited ADP-induced platelet aggregation and had minimal values of aggregation index (20.99) and slope (56.1) (Fig. 5b).

Given that ADP binds to its receptor on the surface of platelets, the binding of ADP to the P2Y1 receptor causes a change in shape and initiates platelet aggregation (primary wave) due to the mobilization of calcium. The

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P2Y12 receptor is considered the main ADP receptor and is responsible for the complete aggregation of platelets through inhibition of adenylate cyclase. In this case, the key link in platelet activation is the mobilization of calcium ions from intracellular depots.

When studying the effect of the studied SMEs on the level of intracellular Ca^{2+} in platelets, it was shown that against the background of ADP (0.5 μg / ml) the compound CC-BOS-122 CC-GSC-63 and CC-BOS-122 at a concentration of 10 μg / μl, differently affected the level of intracellular calcium platelet count. The chelating ability of MSP in relation to calcium ions was studied in intact platelets in comparison with EGTA. As a result of this, it was shown that the compounds CC-GSC-14 and CC-GSC-63, unlike EGTA, do not have the ability to bind calcium ions in intact platelets, which suggests that the compounds CC-GSC-14 and CC - GSC-63 is not a chelator of calcium ions (Fig. 5).

As a comparison drug, the antiplatelet agent heparin, which does not affect calcium homeostasis, was investigated. In order to determine whether the actions of CC-GSC-14 and CC-GSC-63 and CC-BOS-122 are inhibitors of Ca^{2+} entry and mobilization from intracellular depots, we examined them for the level of cytoplasmic concentration of Ca^{2+}. The experiments were performed in two stages both in the presence and in the absence of physiological concentrations of Ca^{2+}.

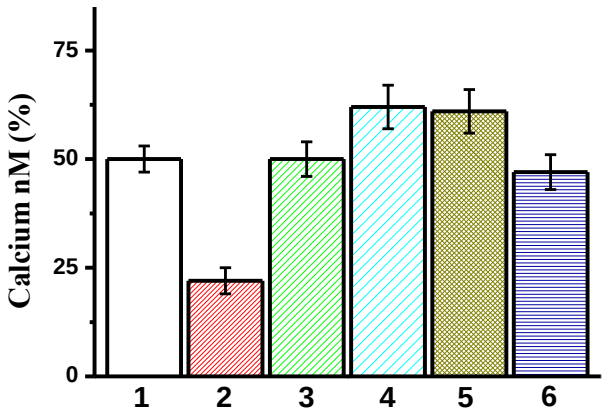

Fig. 5. The effect of compounds CC-GSC-14 and CC-GSC-63 and CC-BOS-122 and EGTA on the binding of calcium ions in intact platelets. 1 - control (platelets were incubated with 5 μM Fura-2 / AM for 30 min at 37 ° C in the absence of Ca^{2+} ions). 2- platelets were incubated with 5 μM Fura-2 / AM for 30 min at 37 ° C in the absence of Ca^{2+} ions in the presence of EGTA. 3-6 heparin and SC-GSC-14 and SC-GSC-63 and SC-BOS-122, respectively.

At the first stage, ADP-induced Ca^{2+} output from cell depots in the presence of physiological concentrations was accompanied by an increase in the content of intracellular Ca^{2+} from the basal level. This indicator was taken for control. Compounds CC-GSC-14 and CC-GSC-63 and CC-BOS-122, added 2 min before induction with ADP, affected the level of intracellular calcium to varying degrees. The compound CC-GSC-14 and heparin at a concentration of 10 μl / ml increased intracellular Ca2 + by 17–19%. Compounds CC-BOS-122 and CC-GSC-63 at a concentration of 10 μl / ml inhibited the yield of intracellular Ca^{2+} by 19–16%, respectively (Fig. 6).

It is possible that the compound SC-BOS-122 leads to a rapid decrease in the concentration of free calcium ions in the platelet cytoplasm, the compound is able to block platelet activation at any stage of the risk of thrombosis.

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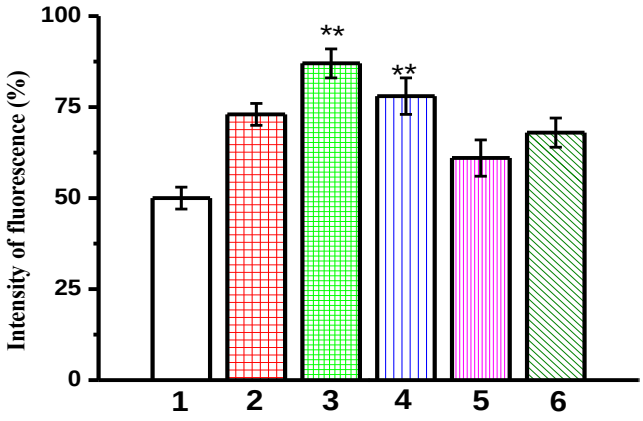

Fig. 6. The effect of heparin and compounds CC-GSC-14, CC-GSC-63 and CC-BOS-122 on the level of intracellular calcium in intact platelets, added 2 min before the induction of ADP.

1. Control-platelets were incubated with 5 μM Fura-2/AM for 30 min at 37° C in the absence of Ca^{2+}.

2 - Effect of ADP on Ca^{2+} output from cell depots.

3-6 - The effect of ADP on the level of intracellular calcium against the background of heparin and compounds SC-GSC-14 and SC-GSC-63 and SC-BOS-122, respectively.

IV. Discussion

The results obtained showed that the studied compounds belong to direct-acting anticoagulants. The results indicate that the studied compounds activating antithrombin III irreversibly inhibit IXa, Xa, XIa, and XIIa factors of the coagulation system, disrupt thrombus formation and inactivate thrombin, and also moderately reduce platelet aggregation. When studying the effect of compounds CC-BOS-122, CC-GSC-63, and CC-GSC-14 on platelet aggregation in healthy human plasma and coronary heart disease, the inhibitory effect of compound CC-BOS-122, in which platelets were induced by ADP and adrenaline, was revealed. Compound CC-BOS-122 at a concentration of 10 μg / μl inhibited ADP-induced platelet aggregation by 50% and reduced max. value (8.09), max. slope (3.95) of the aggregation curve.

Given that ADP binds to its receptor on the surface of platelets, the binding of ADP to the P2Y1 receptor causes a change in shape and initiates platelet aggregation (primary wave) due to the mobilization of calcium. The P2Y12 receptor is considered the main ADP receptor and is responsible for the complete aggregation of platelets through inhibition of adenylate cyclase. In this case, the key link in platelet activation is the mobilization of calcium ions from intracellular depots. It is possible that the compound CC-BOS-122 has the greatest effect on the activity of glycoprotein receptors on the platelet membrane and is of some interest and requires further detailed study of the physicochemical characteristics and mechanisms of their action, which ultimately will allow their use as a heparin-like preparation.

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V. Conclusions

1. The effect of inducers of ADP, adrenaline, collagen and ristomycin on the functional activity of platelets in the blood plasma of a healthy person and coronary heart disease in vitro occurs due to activation of the P2Y12 receptor and the TXA2-dependent pathway in coronary artery disease.

2. The effect of MCP CC-BOS-122 and CC-GSC-63 on ADP, adrenaline-induced platelet aggregation, mediated by their effect on the activity of glycoprotein receptors on the platelet membrane.

3. ICP SC-GSC-14 is not involved in platelet activation and mobilization of calcium ions from intracellular depots. Perhaps its action is due to a direct effect on blood coagulation factors.

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