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Study Of The Effect Of Polysaccharides On Hemostasis

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ABSTRACT

The effect of sulfated polysaccharides on the hemostatic system in conditions in vitro. Platelet-rich plasma was obtained by centrifugation at 200 g for 10 minutes. The remaining citrate blood was further centrifuged at 1500 g for 10 min to obtain platelet-poor plasma. The antithrombin activity of the compounds was evaluated in vitro by their effect on the recalcification time, thrombin, and prothrombin time of human blood plasma stabilized with a 3.8% sodium citrate solution in the ratio of 9:1. In studies conducted on the blood plasma of rats, it was found that the studied compounds, to varying degrees, lengthen the APTT, APTT, prothrombin time. At the same time, anticoagulant activity was established to block one of the factors II, V, X. Polysaccharide exhibit a combined anticoagulant effect in the body, due to which they are classified as anticoagulant and antithrombin agents.

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KEYWORDS

Blood anticoagulants, human plasma, antithrombin, a sulfated polysaccharide.

INTRODUCTION

Currently, for the prevention and treatment of thrombosis in humans, various anticoagulants are used [1; 2]. There are anticoagulants of indirect and direct type of action [3]. The mechanism of action of direct-acting anticoagulants is associated either with direct inhibition of the activity of the thrombin or factor Xa, or with the activation of their plasma inhibitor of the antitrobin [4]. Anticoagulants have a vasodilating effect, improve microcirculation, and increase the fibrinolytic activity of the blood. The use of individual medicinal substances with multidirectional action - anticoagulant, antiplatelet or fibrinolytic in cardiovascular diseases, although it has a positive effect on the state of the hemostasis system, but in case of overdose can lead to an undesirable phenomenon. Factors of the coagulation, anticoagulant (anticoagulant) and fibrinolytic blood systems take part in the hemostatic system. The mechanism of hemostasis includes the interaction between the vessel wall, platelets and blood proteins [5, 6.]. In this system, a balance is very important between the formation of a dense clot, which mechanically closes the damage in the vessel, and the preservation of the functions of the blood, which it can perform only in a liquid state. Violations of the hemostatic system - both excessive thrombus formation and bleeding - can lead to serious pathologies. In order to influence the process of thrombus formation in the bloodstream and its regulation, various anticoagulants based on sulfated polysaccharides are used. Sulfated polysaccharides are unique compounds that affect individual links in the hemostatic system [7, 8]. The mechanism of action of sulfated polysaccharides differs from that of heparin. Sulfated polysaccharides directly inhibit thrombin without the involvement of plasma serine proteinase inhibitors as compared to heparin. No direct inhibition of factor Xa has been identified, sulfated polysaccharides inhibit the internal coagulation pathway in small doses, and in large doses, the external coagulation pathway [9]. It was revealed that sulfated polysaccharides do not possess acute toxicity, but have a broad therapeutic effect. However, the anticoagulant activity of the natural and synthetic sulfated polysaccharides obtained on the basis of cellulose, dextrans and pullulans [10, 11, 12] is lower than the activity of heparin.

The aim of the work was to study the sulfated polysaccharide GSC -32 for individual links of the coagulation system of hemostasis.

METHODS

Experimental biological part

Plasma for research was used without platelets. Platelet-rich plasma was obtained by centrifugation at 200 g for 10 minutes. (Centrifuge OPN-8 (rotor RU180 L, 8,000 rpm) 2007-2008. Russia) The remaining citrate blood was further centrifuged at 1500 g for 10 min to obtain platelet-poor plasma. The antithrombin activity of the compounds was evaluated in vitro by their effect on the recalcification time, thrombin and prothrombin time of rabbit and human blood plasma stabilized with a 3.8% sodium citrate solution in the ratio 9:1 [13]. To isolate platelets used 150 μM NaCl, 2,7 μM KCl,

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0,37 μM NaH2P04 ' 2H2O, 1 μM CaCl2, 5 μM glucose, 10 μM HEPES. In coagulation tests, sulfated compounds were used at a dose of 10-50 μg / mL. Thrombin (1 unit) was used as a control. Prothrombin activity was tested by the Quick method with thromboplastin (Quick). To determine the effect of compounds on thrombosis, it was evaluated by their effect on known hemostasis tests. Thrombin (1 unit) was also used as a control. For experiments using sulfate cellulose 1 mg / 6 mL of H2O. The effect of anticoagulants on the coagulation of human and rabbit plasma in vitro was evaluated using the following tests: activated partial thromboplastin time (APTT) [14], prothrombin time (PT) [15,16], thrombin time (TT) [16], and the ReaClot - Heparin (NPO Rename Russia, Moscow) tests [16] for analysis of the effects on fibrinogen polymerization (for thrombin and buffer, Cypress Diagnostics, Belgium). To study the effect of SC GSC -32 on the anticoagulant activity of rabbit plasma, we intravenously injected the test compound at various doses into the marginal ear vein. After the plasma was collected at different time intervals after SC administration, the clotting time was determined in the APTT/ReaClot-Heparin tests, and the plasma Xa was determined compared with that with heparin.

All coagulation tests (with human plasma) were performed on a single channel coagulometer (CYANCoag, Belgium.CY003, SN:5400439). For evaluation of the anticoagulation potential of the compounds, the effective concentrations in the APTT, PT, TT, and ReaClot tests, which were found on the abscissa of the curves showing the dependence on the concentration of the anticoagulant, were graphically determined. We detected a 2-fold increase in plasma clotting time compared with that of the control, which had no anticoagulants.

Experimental chemical part

In this work, various modified sulphated polysaccharides (MSPs) with different molecular masses and linear compounds obtained via homo- and heterogeneous sulphation, were used.

The modified sulfated polysaccharides studied in this research was cellulose sulfates with different molecular parameters that synthesized, purified and characterized as described in [17,18]. The information regarding the cellulose sulfates is included in the revised manuscript.

RESULTS AND DISCUSSION

The hemostatic system performs a number of vital functions in the body - it maintains the blood in a liquid state, and also prevents the formation of blood clots, prevents bleeding. Normal hemostasis is ensured by the balance between the intense activation of coagulation factors and their inhibitors, and the functioning of the fibrinolytic system. It is known that a violation of the hemostasis system can lead to the formation of a blood clot with subsequent blockage of the bloodstream. At present, to limit the development of such processes, indirect and direct anticoagulants are used - compounds that limit the intensity of coagulation. The well-known anticoagulant heparin is used in emergencies, but its short-lived effect, as well as the ability to cause bleeding, is one of the disadvantages of use. In connection with this problem, research in the field of finding natural or creating new synthetic agents, direct anticoagulants, has not stopped for many years. Work on the study of chemically modified sulfated polysaccharide

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GSC-63 on the human blood plasma coagulation system has been carried out.

To identify the mechanism of the anticoagulant action of GSC-32, a dose-dependent study was conducted in comparison with known anticoagulants. In the process, the anticoagulant effects of GSC-32 on hemocoagulation were investigated in comparison with direct anticoagulants. In studies conducted on the blood plasma of rats (in vitro), it was found that the compounds under study, to varying degrees, lengthen the APTT, APTT, prothrombin time. The thrombin time test is one of the coagulological blood tests, the results of which reflect violations of the final stage of coagulation of the conversion of fibrinogen to fibrin under the action of thrombin. In this case, the rate of formation of a fibrin clot depends mainly on the amount and functional usefulness of fibrinogen and the presence of anticoagulants in the blood. In the study of the effect of GSC-32 on thrombin time, it was revealed that compounds GSC-32 in the presence of 10 mg / mL had practically no effect on thrombin time. However, an increase in the concentration of compounds up to 50 mg / mL led to a gradual lengthening of the thrombin time. However, an increase in the concentration of compounds up to 50 mg / mL led to a gradual lengthening of the thrombin time. Perhaps the lengthening of thrombin time depended on the dose of fibrinogen in the blood plasma. In the opposite case, if GSC-32 was preincubated with fibrinogen, then thrombin was added, while thrombin time was slightly lengthened (Fig. 1).

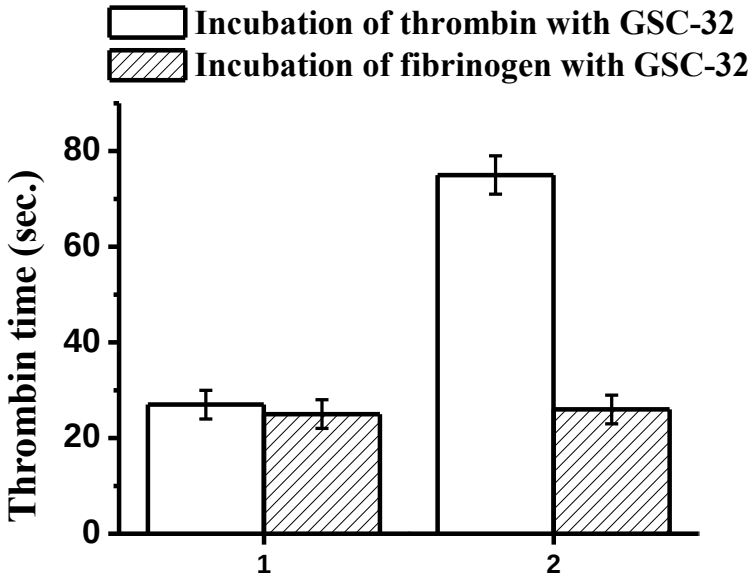

Figure: 1. Investigation of the effect of the GSC-32 polysiccharide on thrombin time.

1 - Control; 2-SC -GSC-32 (50 mg / mL)

The results show that GSC-32 does not affect the conversion of fibrinogen to fibrin, and is possibly associated with the activation of the inhibitory effect of anti-thrombin III on factor Xa, similar to heparin. The mechanism of

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anticoagulant action of sulfated polysaccharides and heparin has a number of differences. The anticoagulant effect of heparin is associated with a direct effect on the blood coagulation system due to the formation of complexes with many factors of hemocoagulation, in particular with antithrombin III and is manifested in the inhibition of I, II and III phases of coagulation. SPs realize an anticoagulant effect, apparently through direct interaction with factors of blood coagulation (with thrombin) and is due to inhibition of the activity of factors of the internal blood coagulation pathway-XI, XII and VIII, while the activity of factors of the external pathway II, V, VII and X is not reliably changes.

In the next experiment, the effect of the compound GSC-32 on the activated partial thromboplastin time was investigated, where the APTT changes depending on different amounts of fibrinogen, prothrombin activity, factors V, VIII, IX, X, XI, XII, in the presence of specific inhibitors - coagulation factors VIII and IX. Prolonged APTT reflects a deficiency or inhibition of coagulation factors (except for VII and XIII).

In experiments in the control of APTT, it was shown that under the action of the compounds, the plasma clotting time corresponded to 20.8 ± 2.0 sec. The action of SC-GSC-32 at concentrations (5-50 μg / mL), dose-dependently prolonged the time of thrombus formation (up to 80-100 sec), lengthened the time of thrombus formation (Fig. 2.).

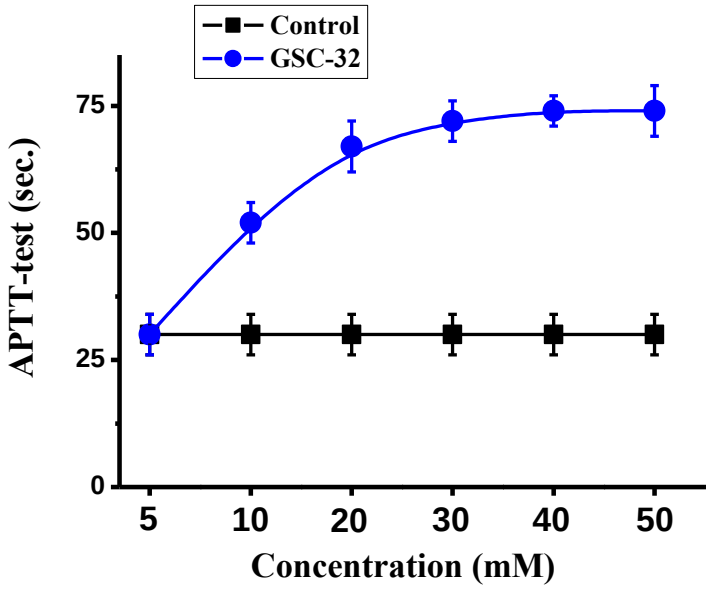

Figure: 2. Investigation of the effect of SC-GSC-32 on the APTT.

It is known that APTT is sensitive to the deficiency of plasma coagulation factors involved in the internal coagulation mechanism (factors XII, XI, IX, VIII) and does not depend on platelet deficiency or functional insufficiency. Based on the results obtained, it can be assumed that GSC-32 affects the factors of the internal blood coagulation pathway, leading to

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a weakening of the formation of a fibrin clot, which may indicate inhibition of the activity of one of the factors XII, XI, IX, VIII. The main method of controlling hemostasis is to determine the duration of prothrombin time. The test is used to detect violations of the activity of factors of the external coagulation pathway. By lengthening the prothrombin time, it is judged that the factors of the external mechanism of prothrombinase formation, its action on prothrombin and the subsequent formation of fibrin are insufficient. This indicator makes it possible to compare the results of coagulation studies and the anticoagulant activity of various compounds. When studying the effect of GSC-32 on prothrombin time, it was found that GSC-32 dose-dependently lengthens the blood coagulation time (Fig. 3.).

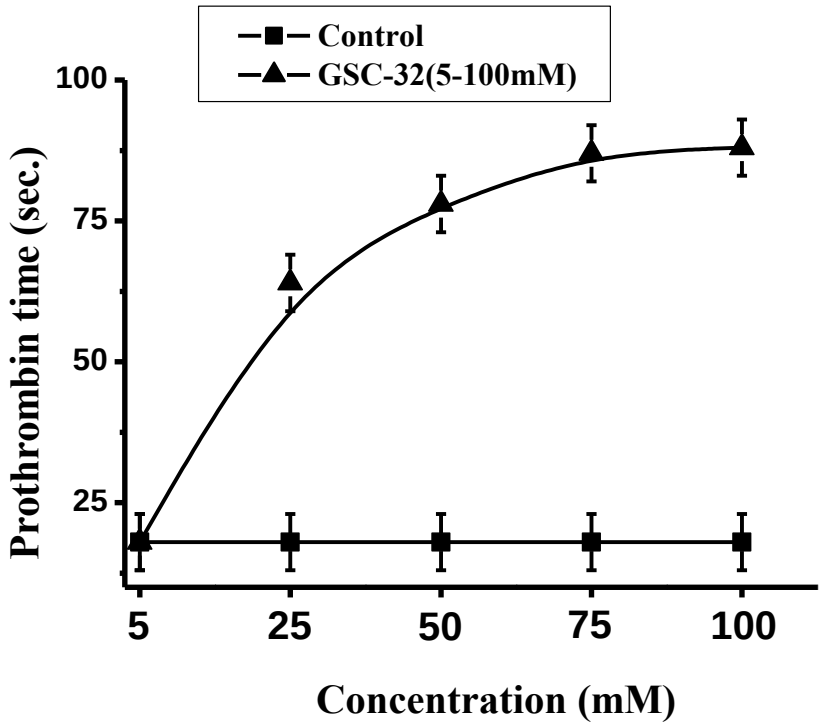

Figure: 3. Effect of GSC-32 on prothrombin time.

By lengthening the prothrombin time against the background of the GSC-32 compound, one can judge about the inhibition of the activation factors of the external coagulation mechanism, that is, inhibition of the activity of V, II and VII factors, the formation of prothrombinase, its action on prothrombin and the subsequent formation of fibrin. The results obtained showed that GSC-32 have a significant effect on factors of the external blood coagulation pathway.

CONCLUSIONS

Thus, a comparison of the obtained results of the APTT test with prothrombin and thrombin time allows us to determine the effect of GSC-

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32 compounds on factors VIII, IX, X, XI, XII of the blood coagulation system. Prolongation of APTT and PT on the background of normal TB reflects inhibition of factors II, V, X. The APTT level does not depend on the number of platelets.

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