Activity of coagulation and fibrinolytic system components in the vein thrombus

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Abstract

Purpose: Behaviour of the vein thrombus is determined by the activity ratio of coagulation factors to factors of fibrinolytic system. The aim of the study is to evaluate activity of some coagulation and fibrinolytic factors in the vein thrombus.

Material and methods: The activity of platelets aggregating factors, tissue factor, thrombin, antithrombins, antiheparin factors, plasminogen activators, plasmin (plasminogen) and antiplasmins of the vein thrombus homogenate was determined using coagulative, fibrinolytic and caseinolytic tests. Retracted blood clot was a compared material.

Results: Tissue factor activity in the vein thrombus was above twofold higher and antiheparin activity was nearly twice higher in comparison to the blood clot. The vein thrombus contains also active thrombin. Plasminogen activators activity in the vein thrombus was twofold higher and activity of plasmin (plasminogen) was threefold higher than in the blood clot. High activity of the tissue factor, substances neutralizing heparin and presence of thrombin intensify the thrombus enlargement. However, the thrombotic tendency may be balanced by a high activity of plasminogen activators and high activity of plasmin (plasminogen).

Conclusions:

1) Vein thrombus is characterized by high activity of tissue factor, presence of active thrombin and high antiheparin activity.

2) High coagulative potential of vein thrombus is balanced to a certain grade by high fibrinolytic potential: high activity of plasminogen activators and high activity of plasmin (plasminogen), as well as absence of antiplasmins activity.

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Introduction

The vein wall during thrombophlebitis shows higher activity of the tissue factor and lower activity of the plasminogen activators than the wall of unchanged vein [1]. Interaction of coagulation factors of the inflammatory changed vein wall with plasma coagulation factors, platelets and leucocytes results in formation of parietal thrombus [2,3]. Further thrombus condition is determined by the relation of coagulation factors activity to activity of fibrinolytic system factors. Activity of the vein thrombus haemostatic system components is still unknown.

The aim of this report is to evaluate the activity of platelets aggregating factors, tissue factor, thrombin, antithrombins, antiheparin factors, plasminogen activators, plasmin (plasminogen) and antiplasmins of the vein thrombus homogenate. Coagulation, fibrinolytic and caseinolytic methods were used to determine the factors mentioned above. Plasma coagulation and fibrinolytic factors, including strategic substrates for the haemostatic system such as blood platelets, fibrinogen and fibrin were the substrates for the vein thrombus haemostatic components. The employed methods allow evaluating the real effect of thrombus haemostatic factors on the coagulation – anticoagulation balance in circulating blood so important in examinations of pathobiochemistry and in diagnosis and treatment of venous thromboembolic disease.

Material and methods

Parietal thrombi from varicose saphenous veins in the course of thrombophlebitis were taken during surgery from 8 patients (5 men and 3 women) aged 33-48 years. Thrombosis of the proximal part of the saphenous vein was an indication for operation. Unfractionated and low molecular weight heparins, as well as

antiplatelets drugs were not administered to patients 7 days before and during the operation. The risk factors of venous thromboembolic disease, such as laboratory indicators of thrombophilia and previous deep veins thrombosis were not found in these patients. The signs of circulatory, liver and kidney dysfunction were also absent. The control material consisted of 8 retracted blood clots obtained *in vitro* from blood collected before the operation from the same patients. Thrombi and clots were stored at -70°C. They were thawed directly before the measurements in water with ice and homogenized in 0.15 mol/l NaCl (1:9 w/v) with the use of flow homogeniser. The supernatant obtained by centrifugation (2700 x g, 30 min, 2°C) was used for the examinations.

Reagents: Palitsch borate buffer [4]; fibrinogen, heparin, plasmin, streptokinase and thrombin, Sigma, USA; hirudin, Hoechst AG, Germany; casein, BDH , England; Folin and Ciocalteau reagent, Merk, Germany; substrate platelets rich plasma (250000 platelets/mm^3) and substrate platelets poor plasma obtained from blood taken into 0.1 mol/l sodium citrate (9:1 v/v) and centrifuged at 2°C (500 x g during 5 minutes and 2700 x g during 30 minutes respectively).

1. Activity of the platelet aggregating factors was evaluated by means of the turbidimetric method [5].

0.1 ml of the homogenate and 0.1 ml 0.15 mol/l NaCl or 0.1 ml 50 μg/ml hirudin was added to 0.9 ml of platelets rich plasma and the time of platelet aggregates appearance manifested by decrease of turbidance was measured. The time elapse was a measure of the aggregating factor activity.

2. Tissue factor activity was evaluated by means of the coagulation method [6].

0.1 ml of the homogenate (in controls 0.15 mol/l NaCl) and 0.1 ml 0.5 mol/l $CaCl_2$ were added to 0.2 ml of platelets poor plasma and the coagulation time at 37°C was measured. Shortening of the coagulation time as compared to the controls was accepted as a measure of the tissue factor activity.

3. Thrombin activity was evaluated using fibrinogen [7].

0.1 ml 0.15 mol/l NaCl or 0.1 ml 1.5 μg/ml of hirudin and 0.1 ml of the homogenate, was added to 0.2 ml 0.3% fibrinogen and the coagulation time was measured at 37°C. The fibrinogen coagulation time was a measure of the thrombin activity.

4. Antithrombin activity was evaluated by means of the coagulation method [7].

0.1 ml of the homogenate (in controls 0.1 mol/l NaCl) and 0.1 ml of thrombin (8 u/ml) were added to 0.2 ml of the platelet poor plasma and the coagulation time at 37°C was measured. Prolongation of the coagulation time as compared to the controls was accepted as a measure of the antithrombin activity.

5. Antiheparin activity was determined with use of heparin-thrombin test [8].

0.1 ml of heparin (2.5 u/ml), 0.3 ml of the platelets poor plasma were added to 0.1 ml of the homogenate (in controls 0.15 mol/l NaCl) and preincubated at 37°C for 5 minutes. The coagulation time was measured after adding 0.1 ml of thrombin (40 u/ml). Shortening of the coagulation time as compared to controls was accepted as a measure of antiheparin activity.

6. Activity of the plasminogen activators was evaluated by means of the euglobulin method [6].

0.5 ml of the platelets poor plasma and 0.25 ml of the homogenate (in controls 0.25 ml of 0.15 mol/l NaCl) were added to 10 ml of cooled distilled water acidized to pH 5.3 by acetic acid. The samples were kept at 2°C for one hour. The sediment obtained by centrifugation (2700 x g, 30 minutes, 2°C) was dissolved in 0.5 ml borate buffer. 0.5 ml of 0.025 mol/l $CaCl_2$ containing thrombin (1 u/ml) was added to the samples and the fibrinolysis time of the formed clot was measured in the temperature of 37°C. Shortening of the fibrinolysis time as compared to the controls was accepted as a measure of the plasminogen activators activity.

7. The plasmin activity (after plasminogen activation by streptokinase) was determined by means of the caseinolytic method [9].

0.25 ml of 0.16 mol/l HCl was added to 0.25 ml of homogenate and incubated at 20°C for 15 minutes. After that 0.25 ml of 0.16 mol/l NaOH, 0.2 ml of borate buffer and 0.05 ml of streptokinase (10000 u/ml) were added and preincubated at 37°C for 15 minutes. Then 1 ml of 2% casein was added and the samples were incubated at 37°C for 60 minutes. The reaction was stopped by adding 1 ml of 15% trichloroacetic acid. The samples precipitated in 0 time were accepted as controls. Tyrosine was determined in the clear supernatant obtained by centrifugation by means of Folin and Ciocialteau reagent. The activated plasminogen content was expressed by the increase of quantity of released tyrosine.

8. Activity of antiplasmins was determined by means of the caseinolytic method [10].

0.25 ml of the homogenate (in controls 0.15 mol/l NaCl) was added to 0.25 ml of 0.1% plasmin and preincubated at 37°C for 15 minutes. After that 0.5 ml of 2% casein was added and incubated at 37°C for 60 minutes. The reaction was stopped by adding 1 ml of 10% trichloroacetic acid. Further procedures were as in point 7. Activity of antiplasmins was expressed by the decrease of released tyrosine as compared to controls.

9. The protein content was determined by means of Lowry method [11].

Mean values ±SD of the obtained results are presented. Results obtained for the vein thrombus and blood clot were statistically analyzed by t-Student test, accepting $p<0.05$ as statistically significant.

Results

Blood platelet aggregating factors are present in about 50% of the vein thrombi. They are not found in the blood clot obtained *in vitro* (*Tab. 1*). Activity of blood platelet aggregating factors is cancelled by addition of hirudin. Tissue factor activity of the vein thrombus is above twofold higher than in the blood clot. Active thrombin is found in about 50% of the vein thrombi. Its activity is cancelled by hirudin. Antithrombin activity is not observed in vein thrombus. Antiheparin activity in the vein thrombus is nearly twofold higher than in the blood clot obtained *in vitro*. The activity of plasminogen activators in the vein thrombus is twofold higher than in the blood clot. Plasmin (plasminogen) activity in the vein thrombus is threefold higher than in the blood clot. Antiplasmins activity in the vein thrombus is absent and it is small in the blood clot. The vein thrombus contains less protein than the blood clot.

Table 1. **Activity of coagulation and fibrinolytic systems components in vein thrombus and blood clot**

Determined factor	Control	Vein thrombus	Blood clot
Activity of platelet aggregating factors, s		127.2±14.6	>10 min
Tissue factor activity, s	120.5±12.3	36.5±4.0*	84.6±9.4
Thrombin activity, s		40.1±4.2	>10 min
Antithrombin activity, s	15.6±1.2	14.5±2.0	15.3±1.4
Antiheparin activity, s	32.0±2.6	18.6±1.2*	23.7±2.4
Plasminogen activators activity, min	225.0±2.04	52.8±6.7*	96.5±12.0
Plasmin (plasminogen) activity, Tyr nmol/ml		182.6±20.4*	48.6±5.8
Antiplasmin activity, Tyr nmol/ml	220.0±21.6	228.6±20.4	210.8±20.9
Protein, mg/ml		15.3±2.4*	28.6±3.8

* difference statistically significant as compared to blood clot ($p<0.05$)

Discussion

The obtained results show the presence of active thrombin in about 50% of the vein thrombi. It is not found in the blood clot obtained *in vitro*. Thrombin in the vein thrombi evokes blood platelets aggregation and converts fibrinogen into fibrin. Hirudin, as a specific inhibitor of thrombin abolishes its aggregating and coagulating activity in the vein thrombus. Thrombin found in the vein thrombus is bound to fibrin, which makes it resistant to inactivating effect of antithrombin III and heparin [12-15]. Thrombin found in the thrombus may contribute to the vein thrombus enlargement. Fibrin bound thrombin may also evoke rethrombosis observed after thrombolytic treatment of thrombi. Occurrence of rethrombosis was observed after thrombolytic treatment of coronary vessels thrombosis [16,17]. So, the simultaneous treatment with thrombolytic drugs and direct thrombin inhibitors, such as hirudin or synthetic inhibitors is reasonable [18].

The high activity of tissue factor and high antiheparin activity of the vein thrombi promote prothrombin activation and active thrombin formation. Migrating cells such as macrophages, granulocytes and blood platelets are the most probable source of the tissue factor activity and antiheparin factors [19,20].

Increased coagulative activity of the vein thrombi is balanced to some extent by marked activity of plasminogen activators, high activity of plasmin (plasminogen), as well as absence of antiplasmins.

Conclusions

Vein thrombus is characterized by high activity of tissue factor, high antiheparin activity and presence of active thrombin.

High coagulative potential of vein thrombus is balanced to a certain grade by high fibrinolytic potential: high activity of plasminogen activators and high activity of plasmin (plasminogen), as well as absence of antiplasmins activity.

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