

CHANGES IN PLATELET FUNCTION IN THE COURSE OF FORMATION OF AN INTRAVASCULAR VENOUS THROMBUS

V. I. Shumakov and A. K. Chepurov

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Experiments on rabbits showed that the period of organization of a venous thrombus and the first 24 h after thrombosis is characterized by a decrease in the number of platelets and in their coagulation, adhesive, and electrophoretic activity and by an increase in their aggregation and sedimentation rate. The changes in platelet function became less marked 2-5 days after thrombosis and were actually characteristic of hemorrhage.

KEY WORDS: venous thrombosis; platelet function.

Clinical and experimental investigations have shown that in intramuscular venous thrombosis marked quantitative and qualitative changes take place in the platelets [1, 3-5, 12]. Frequently, however, the findings are conflicting. Some workers [2, 4, 12], for instance, observed thrombocytosis, increased coagulation and aggregation properties of the platelets, and a decrease in their electrophoretic mobility in intravascular venous thrombosis; others [13, 14] found the opposite changes in platelet function. These contradictions evidently arose because the investigations of the coagulation and physicochemical properties of the platelets were carried out at different times of organization of the venous thrombus. Usually in clinical practice it is impossible to study the dynamics of changes in platelet function at different times of formation of a venous thrombus, and no experimental data on this problem could be found.

This paper describes the study of changes in the coagulation and physicochemical properties of platelets at various times of organization of an intravascular venous thrombus.

EXPERIMENTAL METHOD

Experiments were carried out on 54 rabbits weighing 2.5-3 kg. A method of production of an experimental intravascular thrombus corresponding to clinical conditions was chosen. For this purpose an intravascular thrombus was produced in the femoral vein [6]. By this method operative trauma and mechanical injury to the vein could be completely ruled out. Blood for the study of platelet function was taken before thrombosis and at various times after its production. The coagulation activity of the platelets was estimated by double elastography [9]. The thromboelastogram of platelet-free plasma and of plasma containing 250,000 platelets/mm³ was recorded. The following physicochemical properties of the platelets were investigated: aggregation induced by ADP, recorded on an aggregatometer (EE1); the rate and degree of agglutination [3]; mechanical resistance [8]; osmotic resistance [7]; adhesion of the platelets to a collagen fiber [15]; sedimentation rate [10]; electrophoretic mobility on a cytopherometer (Opton). The platelet count also was determined by phase-contrast microscopy. After the investigations the presence and localization of the thrombus in the femoral vein were determined. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The mean platelet count in the rabbits was $669,000 \pm 28,000$. During organization of the thrombus in the femoral vein the platelet count fell by $277,000 \pm 11,000$ below its initial level ($P < 0.01$). Between 1 and

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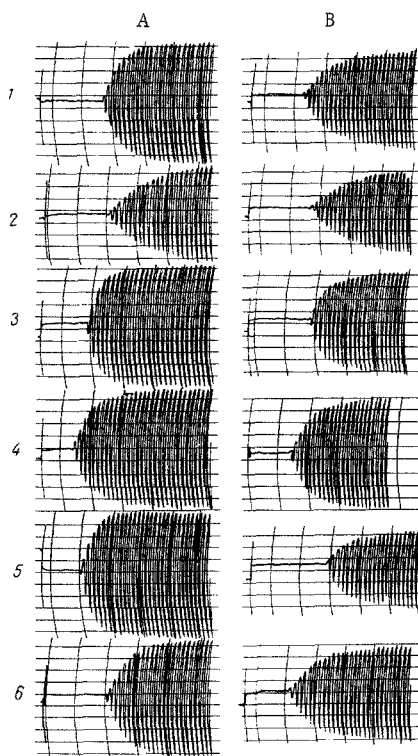


Fig. 1. Thromboelastograms of plasma with (A) and without (B) platelets at various times of organization of the thrombus: 1) background; 2) during thrombus formation; 3) 1-5 min; 4) 3 h; 5) 24 h; and 6) 48 h after thrombus formation.

5 min after thrombosis it rose slightly to $508,000 \pm 22,000$ ($P < 0.05$). The platelet count 3 h after organization of the thrombus was $441,000 \pm 19,000$ ($P < 0.001$), and 24 and 48 h after thrombosis it was $535,000 \pm 14,000$ and $548,000 \pm 26,000$, respectively ($P < 0.05$). The platelet count 3-5 days after organization of the thrombus in the femoral vein varied within its original limits.

During organization of the thrombus in the femoral vein the coagulation activity of the platelets fell (Fig. 1). This was shown by an increase in the total blood clotting time, the reaction time, and the coagulation time and a decrease in the maximal amplitude of the thromboelastogram. The double thromboelastography test showed an increase in the coagulation properties of the platelets 1-5 min after thrombosis, whereas the coagulation activity of platelet-free plasma was within its initial limits. Determination of the coagulation activity of the platelets 3 h after organization of the thrombus showed an even stronger tendency toward hypercoagulation. Later the coagulation activity of the platelets was restored almost to its initial level. Hypocoagulation of the platelet-free plasma was still well marked 24 h after thrombosis.

Investigation of the platelet sedimentation rate in venous thrombosis showed a tendency toward shortening of the sedimentation time within the first 3 h after organization of the thrombus. For instance, 3 h after thrombosis the sedimentation rate of the platelets was increased by 0.14 ± 0.02 over its initial value ($P < 0.01$), after 1-3 days it was within the initial limits, but later it increased and, in particular, on the 4th and 5th days after organization of the thrombus it was 0.93 ± 0.013 and 0.99 ± 0.017 , respectively ($P < 0.01$). During organization of the thrombus and the first 24 h thereafter the adhesive activity of the platelets was reduced by $23 \pm 4\%$ below the initial level. The adhesiveness of the platelets rose sharply 48 h after thrombosis and remained high throughout the rest of the investigation. The maximal increase in platelet adhesion was observed after 3 and 4 days, when it was 39 ± 2 and $31 \pm 3\%$, respectively, above the initial value ($P < 0.05$). The rate of agglutination of the platelets was slowed for 3 h after thrombosis.

The most marked delay in agglutination was observed during organization of the thrombus (by 79 ± 11 sec above the initial value; $P < 0.01$) and during the first 5 min after thrombosis. The agglutination rate of the platelets 1-5 days after thrombosis varied within the initial limits. The number of agglutinated platelets during organization of the thrombus and throughout the investigation was 9-11 more than initially ($P < 0.05$). Determination of the mechanical and osmotic resistance of the platelets during venous thrombosis revealed considerable changes. The greatest changes were found during the first 24 h after thrombosis. Comparative analysis of the rate and degree of aggregation of the platelets under normal conditions and in venous thrombosis revealed substantial differences (Table 1). During thrombosis the aggregation time fell sharply, but later it rose a little. The time of maximal aggregation was considerably lengthened 48 h after organization of the thrombus, but later it varied within its initial limits. Similar changes also were observed on analysis of the degree of aggregation of the platelets. The maximal amplitude of aggregation was not significantly changed. The electrophoretic mobility of the platelets in normal rabbits was $0.811 \cdot 10^{-4} \pm 0.0061 \cdot 10^{-4} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$ ($P < 0.001$). During thrombosis and after organization of the thrombus it fell sharply; the decrease was most marked 5 min after thrombosis, when it was $0.304 \cdot 10^{-4} \pm 0.0023 \cdot 10^{-4} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$ lower than initially ($P < 0.01$). The electrophoretic mobility was still much below normal 5 days after organization of the thrombus.

The results show that in intravascular venous thrombosis marked quantitative and qualitative changes are observed in the platelets. The most important changes are observed during the first 24 h after thrombus formation. Unlike the results obtained by other workers [11, 12], in the present experiments the period of organization of the thrombus and the first 24 h after its formation were characterized by a decrease in

TABLE 1. Dynamics of Platelet Aggregation Indices during Intravascular Venous Thrombosis

Time of investigation	Aggregatometry parameters		
	deg. of aggregation (mm)	time of maximal aggregation (sec)	maximal amplitude (mm)
Background	16±1,4	324±28	3,6±1
During thrombosis	4,5±1	45±12	3,6±1
<i>P</i>	<0,001	<0,01	<0,5
After thrombosis			
1—5 min	14,2±1,9	236±19	3,5±0,8
<i>P</i>	<0,05	<0,05	>0,5
" 3 h	5,1±2,6	176±23	2,7±0,6
<i>P</i>	<0,01	<0,05	<0,05
" 24 h	17,3±2,2	318±34	3,1±1,2
<i>P</i>	>0,5	>0,5	>0,5
" 2 days	33,3±3,1	552±20	2,6±1
<i>P</i>	<0,01	<0,05	<0,05
" 3 days	16,4±1,8	348±14	3,2±1,1
<i>P</i>	>0,5	<0,5	>0,5

Note: Significance of differences (*P*) compared with background.

function became less marked than initially and some of the indices were actually characteristic of hemorrhage. It thus follows that the times of organization of the thrombus must be taken into account and the most precise indices of platelet function must be determined before rational thrombolytic therapy can be instituted.

the number of platelets and in their coagulation activity and adhesiveness. Meanwhile an increase was observed in the aggregation activity of the platelets and in their sedimentation rate. The mechanical and osmotic resistance of the platelets was reduced in venous thrombosis. The coagulation activity of platelet-free plasma, incidentally, was unchanged or was characteristic of blood hypocoagulation. The most constant changes in thrombosis at the different stages of organization of the thrombus were to be found in the electrophoretic mobility which was reduced.

Analysis of the experimental results suggests that in intravascular venous thrombosis the quantitative and qualitative changes in the platelets are phasic in character. The decrease in number of platelets and in their coagulation activity, with a simultaneous increase in their aggregation and sedimentation rate during the first 24 h after thrombosis are evidence of the important role of the platelets in thrombus organization. From 2 to 5 days after organization of the thrombus the changes in platelet

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