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TRANSMISSION ELECTRON MICROSCOPY FOR THE DIRECT ANALYSIS OF FIBRIN CLOT STRUCTURE

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Fibrin polymerization is a crucial process of blood clotting that provides the three-dimension core for the blood thrombus. The main approach of studying fibrin polymerization is still turbidimetry based on light absorption of fibrin solution. It allows to estimate both stages of fibrinogen-fibrin conversion. The initial one, which proceeds without any change of turbidity, reflects the process of protofibril formation, and the second stage of lateral aggregation, is characterized by the rise of turbidity [1]. This method is simple and useful. It allows to estimate the effects of exogenous factors of fibrin polymerization as well as the changes and blood plasma ability to coagulate in pathological conditions.

However, this method has several limitations. First of all, the lag-period of turbidity curve can indicate both inhibition of protofibrils formation and their self-assembly. Also, the final turbidity of the clot is strongly dependent not only on the effectiveness of clotting, but also on the structure of the clot that was obtained. Thick fibrils can be more transparent than the network of thin pathological fibrils. Only confident way to analyze the structure and the mechanisms of formation of fibrin clot is the transmission electron microscopy (TEM) [2].

Aim. The purpose of our study was to compare the structure of clots formed as a result of thrombin-induced fibrin polymerization in the presence or absence of monoclonal fibrin-specific antibodies fragments as factors that change the clot structure. We concentrated on the final stage of fibrin clot formation at maximal turbidity point for every sample.

Methods. Fibrin polymerization was studied by transmission electron microscopy (TEM) of negatively contrasted samples on H-600 Transmission Electron Microscope ("Hitachi",Japan); 1% water solution of uranyl acetate ("Merck", Germany) was used as a negative contrast. For sample preparation, in sterile glass tubes were sequentially added 0.32 mg/mL human fibrinogen, 0.025 M $CaCl_2$ in 0.05 M ammonium formiate buffer (pH 7.9), and a total sample volume was 0.22 mL. The polymerization of fibrin was initiated by the introduction of thrombin at a final concentration of 0.25 NIH/mL. After 180 s, aliquots were taken from the polymerization medium. Each aliquot was diluted to a final fibrinogen concentration of 0.07 mg/mL; 0.01 mL probes of fibrinogen solution were transferred to a carbon lattice, which was treated with a 1% uranyl acetate solution after 2 min. Investigations were per-formed using an H-600 electron microscope at 75 kV. Electron microscopic images were obtained at magnification of 20,000–50,000 [3].

Results. Two monoclonal antibodies fragments were obtained towards the mixture of separated Aα-, Bβ- and γ-chains of fibrinogen. Antibodies fragments that were marked as III-1D and I-4A, had different epitopes within fragment Aα 105–206 of D-region of fibrinogen.

It was shown that addition of antibody fragment I-4A lead to formation of abnormal fibrils that were thinner than in the control sample and were organized in the dense network (Figure). Control sample exhibited the thick fibrils with well-structured classically organized network. The difference between control and I-4A samples demonstrated that antibody I-4A disrupted the structure of polymerized fibrin. In the same time the fibrils obtained in the presence of antibody fragment III-1D were closer to the control ones (Figure).

Discussion. TEM allowed to demonstrate the difference in the action of antibody fragments III-1D and I-4A on thrombin-induced fibrin polymerization. Despite that fact that these antibodies fragments were targeted to the same zone Aα 105–206, they acted on the process in a different

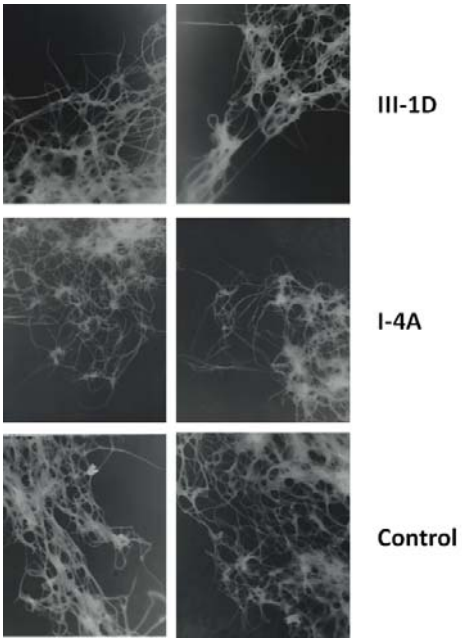

Figure. **Electron microscopy of polymerized fibrin at a point of maximal turbidity:**
III-1D — in the presence of monoclonal anti-Aα105-206 antibody fragment III-1D;
I-4A — in the presence of monoclonal anti-Aα105-206 antibody fragment I-4A;
control — without addition of monoclonal antibodies fragments

manner. This fact allowed us to assume the different functional properties of sites that correspond to the epitope of the studied antibodies fragments.

Conclusions. TEM is an informative method for the study of the fibrin network formation. Its application allows to estimate the disruption in fi brin formation directly. In a combination with turbidity study and other functional tests TEM can provide important information about molecular mechanisms of clot formation.

Key words: fibrin; TEM; fab; polymerization; fibrils; antibody fragment.

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