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BIOCHEMICAL MECHANISMS OF FIBRIN CLOT FORMATION AND SUBSEQUENT LYSIS REGULATION BY PLATELETS

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Introduction. Platelets as key regulators of hemostasis and thrombosis are responsible for dynamic balance between coagulation and fibrinolysis pathways. They form an interface on which important events – thrombin generation, fibrin formation and degradation of blood clot – are initiated, progressed and terminated. Besides, platelets also mediate coagulation and fibrinolysis by supplying a number of coagulation and fibrinolytic proteins as well as their inhibitors. The aim of this study was to investigate the biochemical mechanisms underlying the net effect of platelets on the processes of fibrin clot formation and subsequent lysis.

Methods. Coagulation and lysis of freshly obtained platelet rich plasma (PRP) were monitored using clot waveform analysis assay, by absorbance measurements at 405 nm. Activation of protein C and thrombin generation was estimated with specific chromogenic substrate assay. Flow cytometry analysis was performed to evaluate platelets activation.

Results and Discussion. To investigate platelets impact on the intrinsic coagulation pathway, coagulation of PRP was initiated by calcium chloride (final concentration 8 mM), and the initiation of coagulation by 8 mM calcium chloride with 0.5 nM thrombin was then performed to establish the influence of platelets on terminal reactions of coagulation cascade. Under these conditions, platelets stimulated coagulation in direct proportion to the cell number. Nevertheless, for most of the PRP samples tested, the increase in maximum absorbance was accompanied by its subsequent decrease, which indicates the conversion of the polymerization of fibrin into its spontaneous lysis, even without rt-PA addition. To prove the contribution of the plasminogen/plasmin system to spontaneous degradation of PRP-derived clot, PRP was preincubated with 6-aminohexanoate (AHA), a blocker of lysine-binding sites in plasminogen. Addition of 5 and 10 mmol/l of AHA to PRP prior to clotting caused complete inhibition of spontaneous dissolution of the clot. The inhibition of fibrinolysis after the addition of anti-PC antibodies in PRP (final concentration 18 μg/ml) and the activation of protein C (confirmed by specific chromogenic substrate assay) on platelets suggests certain role of protein C pathway in platelets regulation of hemostasis. Additionally, platelets accelerated thrombin production by prothrombin complex. These results were supported by flow cytometry analysis, which showed that under thrombin activation, a population of platelets with high level of PS and PI signal was formed, that provide them procoagulant properties due to the binding and activation of coagulation cascade proteins. The impact of prothrombin complex on plasminogen binding and activation can be another possible phase on which coagulation and subsequent lysis can be regulated by platelets.

Conclusions. The obtained data gives the evidence that platelets can selectively regulate coagulation and fibrinolysis, thereby adapting the local hemostatic balance, the size and lifetime of the fibrin clot to formation of physiological hemostatic plug or thrombus. Modulation of plasminogen binding and activation, as well as the activation of protein C on platelets surface can be one of the possible mechanisms of such regulation.

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