

Identification of a Unique Platelet Contractile Mechanism Regulating Thrombus Stability

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Aim

To gain insight into the mechanisms regulating thrombus formation and stability under flow.

Method

Thrombus stability was examined using a well defined *in vitro* flow-based thrombosis assay, independent of blood coagulation. 3D analysis of thrombus volume was performed using confocal microscopy.

Results

Real-time analysis of thrombi forming on a collagen matrix, but not on a VWF or fibrinogen matrix, revealed a distinct stage of thrombus development, which we have termed thrombus contraction. Thrombus contraction was apparent during the formation of large platelet aggregates on a collagen substrate and typically occurred over the first 5 minutes of thrombus development. Real-time 3-D analysis of forming thrombi by confocal microscopy revealed that thrombus contraction was associated with a 30-40% reduction in thrombus size (n=12). Thrombus retraction was shear-dependent, with the rate of retraction increasing as a function of increasing blood flow rates. This retraction was accompanied by consolidation of the thrombus core, suggesting that thrombus contraction may play a role in promoting thrombus stability. Inhibiting the platelet contractile mechanism by pretreating platelets with the specific myosin IIa inhibitor, blebbistatin revealed an important role for the actinomyosin cytoskeleton in promoting clot retraction and thrombus stability. Furthermore, analysis of a range of signalling cascades involved in regulating platelet cytoskeletal function uncovered a major role for the Rho kinase signalling pathway in regulating thrombus contraction and stability.

Conclusions

These studies define a unique platelet contractile mechanism regulating thrombus stability under flow. This contractile mechanism is distinct from classical fibrin clot retraction in that the former is fibrin-independent, only occurs with larger thrombi and is regulated by shear. Moreover, unlike fibrin clot retraction, platelet thrombus contraction involves Rho kinase, a pathway not previously implicated in platelet contractile functions, raising the possibility that RhoA/Rho kinase inhibitors may represent new approaches to modulate thrombus stability *in vivo*.