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"Protein S Facilitates Clot Retraction Through Tyro3 Signaling on Platelets"

Abstract

Objective: Clot retraction is a critical step in hemostasis. Our data suggest that Protein S (PS), a known anticoagulant, is also required for clot retraction by binding to TAM family tyrosine kinase receptors (Tyro3, Axl, and Mer) and initiating a signaling cascade. While PS is primarily secreted by the liver, a small fraction is derived from platelets. Based on preliminary findings indicating that platelet-derived PS is essential for clot retraction, we hypothesize that platelet PS mediates this process, with Tyro3 being the principal TAM receptor involved.

Methods: Citrated blood from healthy volunteers was used to isolate platelets by centrifugation, which were then resuspended in PS-deficient plasma. Reaction mixtures contained platelets in PS-deficient plasma (200 µL), HEPES-Tyrode buffer (745 µL), and red blood cells (5 µL). Experimental groups included: control, 500 nM Tyro3 inhibitor, 100 nM Axl/Mer inhibitor, 300 nM exogenous PS, and 300 nM PS with 500 nM Tyro3 inhibitor. Clotting was initiated by adding 50 µL thrombin (20 U/mL). Clot retraction was monitored over time by imaging and weighing the clots.

Results: Control samples, containing only platelet-derived PS, demonstrated normal clot retraction, whereas retraction was abrogated in platelet-poor plasma treated with PS-neutralizing antibody, confirming the role of platelet PS. Inhibition of Tyro3 significantly impaired clot retraction, resulting in minimal reduction in clot mass and size over time. In contrast, Axl/Mer inhibition produced only a modest effect. Addition of exogenous PS enhanced clot retraction, an effect reversed by Tyro3 inhibition.

Conclusion: Our findings demonstrate that platelet-derived PS is essential for clot retraction and acts through TAM receptors, with Tyro3 playing the predominant role. These results highlight a novel platelet-mediated mechanism of clot retraction involving PS–Tyro3 signaling.