Extracellular Actin Potentiates Platelet Aggregation Through Collagen Activation Pathway

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BACKGROUND

Trauma remains a leading cause of morbidity and mortality. Two deadly complications of severe trauma are coagulopathy and microvascular blood flow derangement. The mechanisms of each are not completely understood, though tissue damage and diffuse activation of coagulation likely play roles. Tissue damage from severe trauma can lead to massive release of the cytoskeletal protein actin into the blood. Extracellular actin has complex effects on blood clotting, and excessive actin release is associated with platelet dysfunction after severe trauma. This implicates extracellular actin as a possible contributor to the changes in coagulation and microcirculation seen after trauma. Gelsolin is an actin-scavenging protein that circulates in the plasma and caps actin filaments, solubilizing them and allowing them to be cleared from the blood. However, the specific effects of actin and gelsolin on platelets and the pathways involved remain unknown.

OBJECTIVE

Determine the effects of exogenous administration of human actin and gelsolin on platelet aggregation in healthy donor blood and identify possible mechanistic pathways involved.

METHODS

Healthy donor whole blood in 3.2% sodium citrate was combined with either a saline control or recombinant human skeletal muscle-derived actin (final concentration 200 nM) and allowed to incubate for 5 min. Samples were then activated with either 10 mM adenosine diphosphate (ADP) or 2 mg/mL collagen. The platelet aggregation response was then measured by impedance aggregometry. Each pair of control and actin conditions was run simultaneously. The impedance area under the curve (AUC) was compared between control and actin groups under each activation condition using a paired t-test with significance at $p<0.05$. An actin dose-response curve was generated by activating whole blood samples containing increasing concentrations of actin (0 nM, 50, 200, 1000 nM) with 2 mg/mL of collagen.

The effect of gelsolin was measured by combining whole blood with either actin (200 nM) alone or actin and gelsolin (20 nM), incubating for 5 min, then testing aggregation in response to collagen (2 mg/mL) activation.

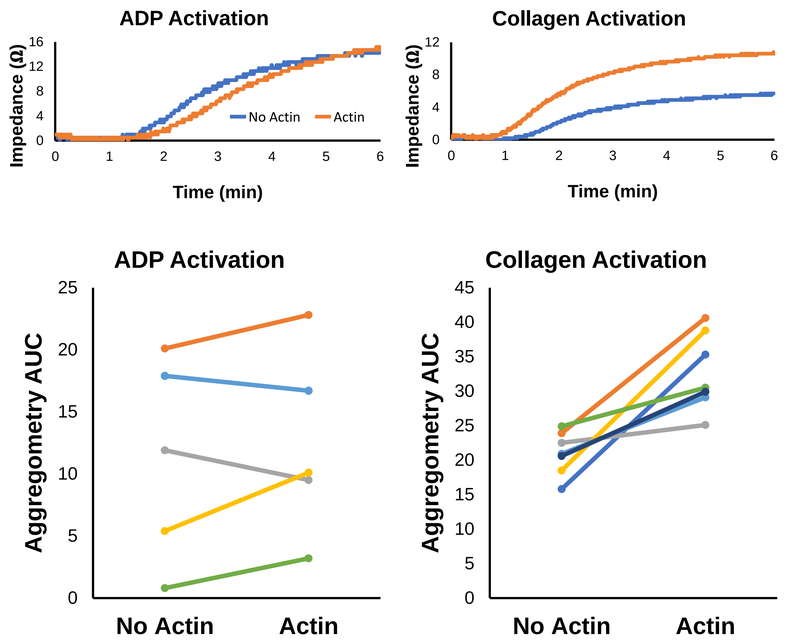

Figure 1. Effect of exogenous actin on platelet aggregation. (Top) Representative aggregometry tracings in response to ADP and collagen activation in the absence or presence of exogenous actin. (Bottom) Summary of aggregometry responses (connected points run simultaneously). ADP, adenosine diphosphate; AUC, area under the curve.

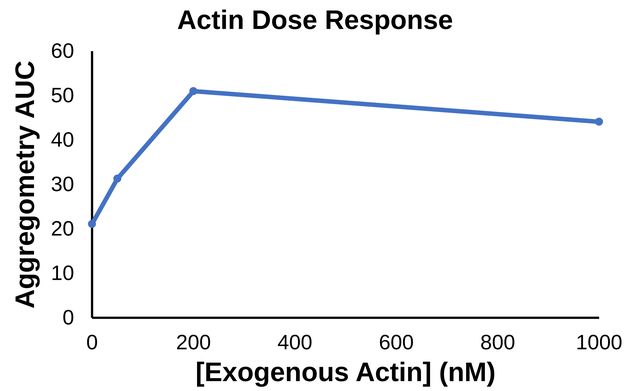

Figure 2. Dose-response relationship between the concentration of added exogenous actin and aggregometry response after collagen activation. AUC, area under the curve.

RESULTS

The AUC in response to ADP was no different between the control and actin groups (mean 11.2 vs. 12.5, p=0.400, n=5). The AUC in response to collagen was significantly higher in the presence of actin compared to control (21.0 vs. 32.8, p=0.005, n=7).

The AUC response to collagen increased with higher concentrations of actin, but plateaued after 200 nM, suggesting a saturation for actin's effect on platelet aggregation.

The AUC response to collagen was not significantly lower when gelsolin was added in the presence of actin, though repetitions were low (34.2 vs. 18.1, p=0.058, n=4).

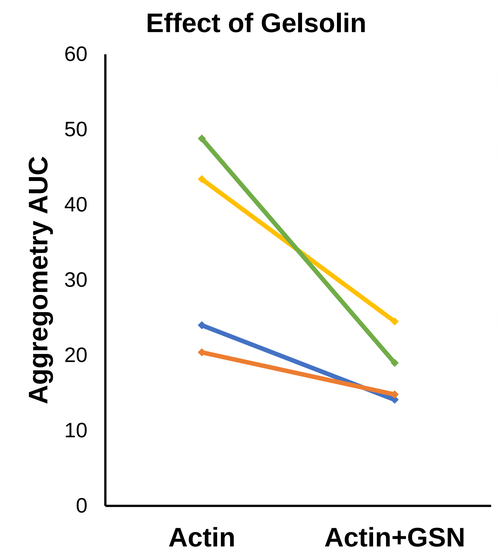

Figure 3. Effect of exogenous gelsolin on platelet aggregation in the presence of exogenous actin. Samples activated with collagen. Connected points were run simultaneously. AUC, area under the curve; GSN, gelsolin.

CONCLUSIONS

The addition of exogenous skeletal muscle-derived actin increases the platelet aggregation response through the collagen but not the ADP activation pathway. Gelsolin appears to reverse actin's effect in the collagen activation pathway. This suggests that extracellular filamentous actin release after injury could cause an overactivation response by platelets and contribute to derangement of microvascular blood flow and derangement of primary hemostasis after injury.

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